Flu A qcLAMP kit

CE IVD

REF 000056

Σ 100 tests

INSTRUCTIONS FOR USE (IFU)

For *in vitro* diagnostics use

For Professional use only

Not for Self-Testing

BIOPIX-T

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PREAMBLE

Indication to EU IVD Directive 98/79/EEC
1. Product Category: IVD Kit
2. Product Name: Flu A qcLAMP kit
3. Product Catalogue Number: cat. no.#000056
4. Purpose of use: See Section “Intended Use”

Inquiries and customer service (A/S)
Send us an e-mail (info@biopix-t.com) to inquire about the product.

Contents

Intended Use

The Flu A qcLAMP kit is a molecular *in vitro* diagnostic test that is intended to be used for the detection of Influenza A viral RNA in association with the Pebble qcLAMP Point-of-Care Platform.

The Flu A qcLAMP kit does not require RNA extraction and can be used with nasopharyngeal and oropharyngeal swab specimens, collected from individuals suspected of Flu. The kit can also be used with extracted RNA.

The Influenza A RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of Influenza A RNA.Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results should be considered as potentially negative and do not preclude Influenza A infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Flu A qcLAMP kit is intended to be used by trained personnel or healthcare professionals (experts), who are proficient in performing tests on the Pebble qcLAMP Platform. It is not intended to be used for self-testing.

Product description

The Flu A qcLAMP kit is a Nucleic Acid Amplification Test (NAAT) kit based on the real-time colorimetric LAMP method (qcLAMP) for the detection of H1N1 and H3N2 Influenza A HA-gene RNA target. The Flu A qcLAMP kit does not require RNA extraction and can be used directly with nasopharyngeal and oropharyngeal swab specimens. Alternatively, the kit can also be used with extracted RNA.

The kit includes a 2X Enzyme mix which contains a mixture of Bst polymerase and thermostable reverse transcriptase, optimized reaction buffer, Mg^{2+} and dNTPs. It further comprises a separate 5X Flu A Primer mix which contains Influenza A specific primers and hydroxynaphthol blue (HNB) indicator, and a 5X Control Primer mix for amplifying a human endogenous target (RNase P) and hydroxynaphthol blue (HNB). The supplied 2X BPX sample buffer neutralizes common sample inhibitors for direct crude sample amplification and detection. The kit also includes Mineral oil, which helps to prevent cross-contamination and minimize evaporation.

Each kit is sufficient for 100 reactions. Tests are performed in the Pebble qcLAMP Platform, which controls the reaction temperature, timing and facilitates the real-time digital colorimetric analysis of the amplification reactions. Total duration of the test in the Pebble qcLAMP Platform is **20 minutes** for assessing a negative result. Time-to-positive can range between 8 and 20 minutes depending on the initial target concentration.

Kits contents and components

The Flu A qcLAMP kit contains the following components for performing **100 qcLAMP tests/reactions**:

Flu A qcLAMP kit components

Component	Quantity	Volume	Description	
2X Enzyme mix	1 tube	1.25 mL	Colourless solution	Black cap
5X Flu A Primer mix	1 tube	0.5 mL	Purple solution	Purple cap
5X Control Primer mix	1 tube	0.25 mL	Purple solution	Green cap
Nuclease-free water	1 tube	1.0 mL	Colourless solution	White cap

2X BPX sample buffer	6 tubes	1.8 mL	Colourless solution	Neutral cap
Mineral oil	1 tube	1.8 mL	Colourless solution	Blue cap

Storage and handling conditions

On arrival, the components of the kits should be stored in the original packaging at -20°C.
During the preparation of the test reactions, all materials except Mineral oil must be kept on ice or in a refrigerated storage container (0-4°C). Mineral oil during the handling of materials can remain at room temperature.

Improper handling of the kit components can lead to deterioration of the Flu A qcLAMP kit and as a result to false results!

Before the first use of the Flu A qcLAMP kit, it is recommended to aliquot part of the initial volume of the reagents **2X Enzyme mix** and the **5X Flu A Primer mix** to reduce the risk of deterioration of the kit due to multiple freeze-thaw cycles.
Avoid more than 2 freeze-thaw cycles.

Shelf-life of the kit

The kit is stable for 12 months at -20°C.

Additional Material & Equipment Required but Not Supplied

Materials and components required for detection of Influenza A that are not included within the kit are:

For use with extracted RNA or crude samples:

A. Kits for the collection and transportation of specimens with viruses that affect the upper respiratory specimens
B. The Pebble qcLAMP Point-of-Care Platform (BIOPIX-T)
C. Positive (synthetic RNA template or plasmid) and negative controls (Genomic DNA or RNase free water).
D. Other Equipment and Materials:
    1 Adjustable calibrated pipettes. (10 µL,20 µL, 200 µL and 1000 µL).
    2 Sterilized pipette filter tips (10 µL, 20 µL, 200 µL and 1000 µL).
    3 Multiply-Pro PCR single tube, 0.2 mL from SARSTEDT Cat. No. 72.737.002 (DNA-free, DNase-/RNase-free, PCR Inhibitor-free) or any other equivalent PCR single tube, 0.2 mL with the following specifications:
        Specifications: safe lock, high-profile mini-PCR tubes.
        Dimensions: Height with lid: 21.6mm-21.7mm, outer diameter (OD): 5.9mm-6.1mm.
    4 PCR tubes of 1.5 or 2 mL maximum volume.
    5 DNA and RNA degradation solutions such as DNAZap™, RNAZap™, 10% bleach (1:10 dilution of commercial 5.25-6.0% sodium hypochlorite) or equivalent.
    6 Protective equipment (disposable powder-free gloves and laboratory coat).

For use with extracted RNA only:

E. RNA extraction kits and tools for extraction of total RNA from clinical samples.

Warnings and Precautions

1. For *in vitro* diagnostics (IVD) use.
2. The Flu A qcLAMP kit is certified only for the detection of nucleic acids from Influenza A, not for any other viruses or pathogens.
3. Reagents should be aliquoted into smaller volumes if not used directly to avoid multiple freeze thaw cycles.
4. Specimen processing should be performed following biosafety level 2 (BSL-2) or higher guidelines.
5. Do not open the tubes after the end of qcLAMP reactions to avoid contamination of the area with DNA amplicons.
6. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where reagents are present and human specimens are handled.
7. The use of Flu A qcLAMP kit and data evaluation is restricted to trained personnel only.
8. Do not use any Flu A qcLAMP kit components beyond their expiration date.
9. The Flu A qcLAMP kit has been optimized to be used with the Pebble qcLAMP Platform.
10. Before processing the sample, please check the turbidity and viscosity of the sample. Turbid and viscous samples can influence the reaction and therefore the results. In case of very turbid samples, we recommend 1:10 or 1:100 dilutions of swab samples before proceeding with testing. However, this action will also lower the limit of detection (LOD) of Flu A qcLAMP test.
11. Dispose of any unused and used reagent, container, and human specimens in accordance with federal, state, and local regulations.
12. Wear appropriate personal protective equipment (e.g., gloves, coat, eye protection) when handling clinical specimens.

The following warnings should be considered when using the kit:

Warning	Cause	Recommendation
The initial color of the 5X Flu A Primer mix and 5X Control Primer mix is not purple.	Improper storage of the kit.	Store kit at -20°C.
	Kit reagents underwent too many freeze-thaw cycles.	Minimize the number of freeze-thaw cycles.
The initial color of the reaction is sky blue (before been placed in the Pebble qcLAMP Platform).	The reaction was not properly prepared.	Ensure the proper mixing of the reagents and the accuracy of the pipetted volumes. Mix intensively the component 2X Enzyme mix, 5X Flu A Primer mix and 5X Control Primer before the preparation of the test and after the preparation of the test and before placing it in the Pebble qcLAMP Platform.
	Improper storage of the kit.	Store kit at -20°C.
	Kit used after the expiration date.	Use before the expiration date.
	Kit reagents underwent too many freeze-thaw cycles.	Minimize the number of freeze-thaw cycles.

Positive control reactions do not show amplification.	Kit reagents underwent too many freeze-thaw cycles.	Minimize the number of freeze-thaw cycles.
	Improper storage of the kit.	Store kit at -20°C.
	Kit used after the expiration date.	Use before the expiration date.
	Reaction was not prepared correctly.	Ensure the proper mixing of the reagents and the accuracy of the pipetted volumes. Mix intensively the component 2X Enzyme mix, 5X Flu A Primer mix and 5X Control Primer before the preparation of the test and after the preparation of the test and before placing it in the Pebble qcLAMP Platform.
	No RNA was added.	Ensure that sample is added in the reaction.
	Positive RNA control has been degraded.	Avoid multiple freeze-thaw cycles and aliquot reagents appropriately.
	Kit reagents contaminated by RNases.	Use solutions (e.g., ethanol, chlorine solution, detergent) to clean the surfaces and the devices. Use protective equipment. Replace reagent stocks with new materials.
RNA Extracted samples do not show amplification.	Extraction process was not properly performed.	Use 5X Control Primer mix for the verification of the extraction process. If it does not show amplification, repeat the extraction process
	Viral load is below the limit of detection.	Negative results do not preclude Influenza A infection and should not be used as the sole basis for patient management decisions. Sampling and testing should be repeated at least after 24 h.
	Reaction was not prepared correctly.	Ensure the proper mixing of the reagents and the accuracy of the pipetted volumes. Mix intensively the component 2X Enzyme mix, 5X Flu A Primer

		mix and 5X Control Primer before the preparation of the test and after the preparation of the test and before placing it in the Pebble qcLAMP Platform.
Crude samples do not show amplification.	Crude samples contain mucus or blood.	Reduce viscosity by diluting swab samples 10× or 100× before proceeding, or retake swab sample.
	Viral load is below the limit of detection.	Negative results do not preclude Influenza A infection and should not be used as the sole basis for patient management decisions. Sampling and testing should be repeated at least after 24 h.
	Reaction was not prepared correctly.	Ensure the proper mixing of the reagents and the accuracy of the pipetted volumes. Mix intensively the component 2X Enzyme mix, 5X Flu A Primer mix and 5X Control Primer before the preparation of the test and after the preparation of the test and before placing it in the Pebble qcLAMP Platform.
Negative reactions show amplification.	Sample contamination with RNA.	Never open reaction tubes after amplification. Only use tubes with safe lids and that have not been used before. Use DNA degradation solution to clean the working space and equipment or 10% chlorine bleach solution. Replace reagent stocks with new materials. Change pipette tips between samples.
	Improper storage of the kit.	Store kit at -20°C.
	Kit reagents underwent too many freeze-thaw cycles.	Minimize the number of freeze-thaw cycles.

	Non-template amplification.	All reactions should be set up and kept on ice prior to amplification. Run the qcLAMP reactions immediately after preparation.

Preparation of the reagents

All reagents are ready to be mixed and used without prior preparation.
Before the first use of the Flu A qcLAMP kit, it is recommended to aliquot part of the initial volume of the reagents **2X Enzyme mix** and the **5X Flu A Primer mix** to reduce the risk of deterioration of the kit due to multiple freeze-thaw cycles.
Avoid more than 2 freeze-thaw cycles.

Sample Collection, Handling and Preparation before testing

It is recommended to use all opened reaction tubes, as soon as possible. Use the kit components before the expiration date following the manufacturer's recommendations. It is very important to use tools and reagents free from RNases. In addition, it is recommended to carry out any preparation steps in areas free from nucleases, only using pipettes with filter tips.

To be followed for testing with extracted RNA:

Sample collection process:

1. Collect nasopharyngeal and oropharyngeal swab specimens in any solubilizing solution.
2. Remove 200 µL of the collected sample and perform RNA extraction. Elute extracted RNA in 30 to 50 µL of RNase free water and place immediately on ice or store at -20ºC.

To be followed for testing with a crude sample:

To perform a test without RNA extraction, specific nasopharyngeal and/or oropharyngeal swab specimens should be solubilized in specific viral transport media solutions (VTM). Only **COMPATIBLE** viral transport medium **CAN** be used in combination with Flu A qcLAMP kit (Table 1). **NON** compatible viral transport medium **SHOULD NEVER** be used in combination with Flu A qcLAMP kit (Table 2).

Table 1: Compatible viral transport medium

Compatible viral transport medium	
Brand	**Description**
Saline solution, 0.9% in water	Sodium chloride solution
CITOSWAB®	Viral Transport medium –VTM-, 3mL).
Improviral™	Viral Preservative Medium –VPM-, 3mL
Liofilchem®	Viral Transport Medium –VTM-, 3mL
Bioprepare microbiology	Virus Transport Medium (GLYE)
Sansure Biotech	Sample Storage Reagent (REF.No. X1002E)

Table 2: NON-Compatible viral transport medium

NON compatible viral transport medium	
Brand	**Description**

Biocommma	Virus transport and preservation medium (Inactivated)
MWE	Virus transport medium using Virocult
LITUO	Disposable Virus Sampling Kit
Zymo DNA/RNA Shield Reagent	Transport and storage medium
Biobase China VTM Swab Sample Collection	Disposable virus sampling tube kit

Sample collection should be done as following:

1. Collect nasopharyngeal and oropharyngeal swab specimens in a solubilizing solution.
2. Remove 100 µL of the collected sample and mix well with 100 µL of 2X BPX sample buffer solution. Use immediately.

Test procedure

Sample preparation must be followed prior to the testing procedure. Keep the prepared samples on ice until use.

Keep on ice or in an ice box the Flu A qcLAMP kit tubes (2X Enzyme mix, 5X Flu A Primer mix, 5X Control Primer mix, Nuclease-free water) and the reactions during preparation. Mineral oil could be kept at room temperature during the preparation of the tests.

A. To be followed with extracted RNA samples:

1. Before preparing a mix, **intensively** pipette up and down the solution of 2X Enzyme mix and the solution 5X Flu A Primer mix.

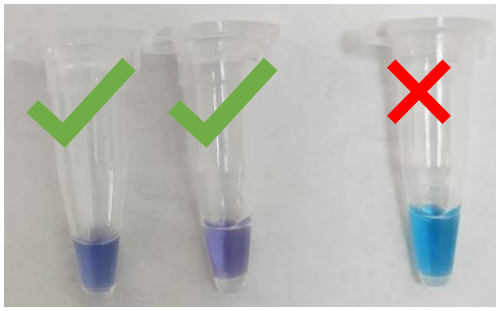
2. Prepare one or more tests as specified in the following table and place on ice. The preparation of a 25 µL for the detection of Influenza A RNA into a 0.2 mL tube (it is not included in the kit) is performed according to the following instructions. For the preparation of 6 samples is preferable to prepare a master mix. Before the addition of the 2 µL extracted RNA, aliquot the mix into 6 different tubes with total volume of 23 µL in each one of them. Then, add 2 µL of extracted RNA from each sample to each different tube. Add the reagents to each tube in the following order (Table 3).

Table 3: Set up of the sample(s) using extracted RNA

Reagent	Volume per reaction (µL)	Volume per 6 reactions (µL)
2X Enzyme mix	12.5	75
5X Flu A or Control Primer mix	5	30
Nuclease-free water	5.5	33
Extracted RNA	2	2 in each tube
Total volume	25	150

Total volume per one reaction should be 25µL.

Note! The initial color of the 25 µL qcLAMP reaction should be purple or deep blue (see figure below – left tube). If the color is sky blue (see figure below – right tube) do not perform the test. For more information consult the warnings and precautions section.



3. Add carefully **15 µL** of mineral oil at the side of each tube and wait approximately 30 sec until it forms a layer over the qcLAMP reaction. Make sure the oil is not mixed with the reaction.

4. Place the reactions in the Pebble qcLAMP Platform and perform the tests by following the instructions for use of the Pebble qcLAMP Platform. The maximum number of samples that can be analyzed simultaneously is 6.

For the verification of a properly performed RNA extraction, the above procedure can be repeated for the detection of a human endogenous target (internal control, IC), the RNase P gene by replacing the 5X Flu A Primer mix with the 5X Control Primer mix. Use the 5X Control Primer mix solution to perform this test. In this case, follow steps 1-4 replacing the 5X Flu A Primer mix tube with the 5X Control Primer mix tube (Table 3). This test is optional and can be performed for each sample separately.

Note! Positive (synthetic RNA template or plasmid) and negative controls (Genomic DNA or Nuclease-free water) should be used during the sample's analysis or separately, to check the quality of the kit ingredients. Positive or negative controls are not included in the kit.

B. To be followed for crude sample direct detection:

1. Before the preparation of the mix, **intensively** pipette up and down the 2X Enzyme mix and the 5X Flu A Primer mix.

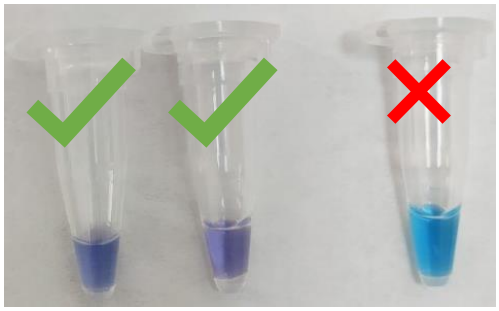
2. Prepare one or more tests as specified in the following table and place on ice. The preparation of a 25 µL for the detection of Influenza A RNA into a 0.2 mL tube (it is not included in the kit) is performed according to the following instructions. For the preparation of 6 samples is preferable to prepare a master mix. Before the addition of the 5 µL crude sample mixed with 2X BPX buffer, aliquot the mix into 6 different tubes with total volume of 20 µL in each one of them. Then, add 5 µL of crude sample mixed with 2X BPX buffer from each sample to each different tube. Add the reagents to each tube in the following order (Table 4):

Table 4: Set up of the sample(s) using crude sample

Reagent	Volume per reaction (µL)	Volume per 6 reactions (µL)
2X Enzyme mix	12.5	75
5X Flu A or Control Primer mix	5	30
Nuclease-free water	2.5	15
Crude sample mixed with 2X BPX buffer	5	5 to each tube
Total volume	25	150

Total volume per one reaction should be 25µL.

Note! The initial color of the 25 µL qcLAMP reaction should be purple or deep blue (see figure below – left tube). If the color is sky blue (see figure below – right tube) do not perform the test. For more information read the warnings and precautions section.



3. Add carefully 15 µL of mineral oil at the side of each tube and wait approximately 30 sec until it forms a layer over the qcLAMP reaction. Make sure the oil is not mixed with the reaction mixture.

4. Place the reactions in the Pebble qcLAMP Platform and perform the tests by following the instructions for use of the Pebble qcLAMP Platform. The maximum number of samples that can be analyzed simultaneously is 6.

Interpretation of the Results

The Pebble qcLAMP Platform employs a mini digital camera for monitoring in real-time the transition through various color shades during colorimetric LAMP amplification. The camera collects non-calibrated images at predefined time intervals (10 sec interval) and automatically extracts the red, green, and blue (RGB) channel values.
Color change is expressed as color index units (pixels) on the Y-axis of a real-time curve that is displayed on the screen of a smart device. Up to 6 (six) curves can be displayed simultaneously.
The duration of a test is 30 min. Evaluation of the results should be performed by the end-user (Please note that this is a test for professional use only).

Positive test results

The specific time-point at which a change in the slope of the real-time curve occurs (color index units increase) corresponds to the “time-to-positive” result (red line). This is the point at which the arrow shows.
When a sample is positive for any target, the slope of the curve **will change** to positive values as shown in the Figure 1 below.

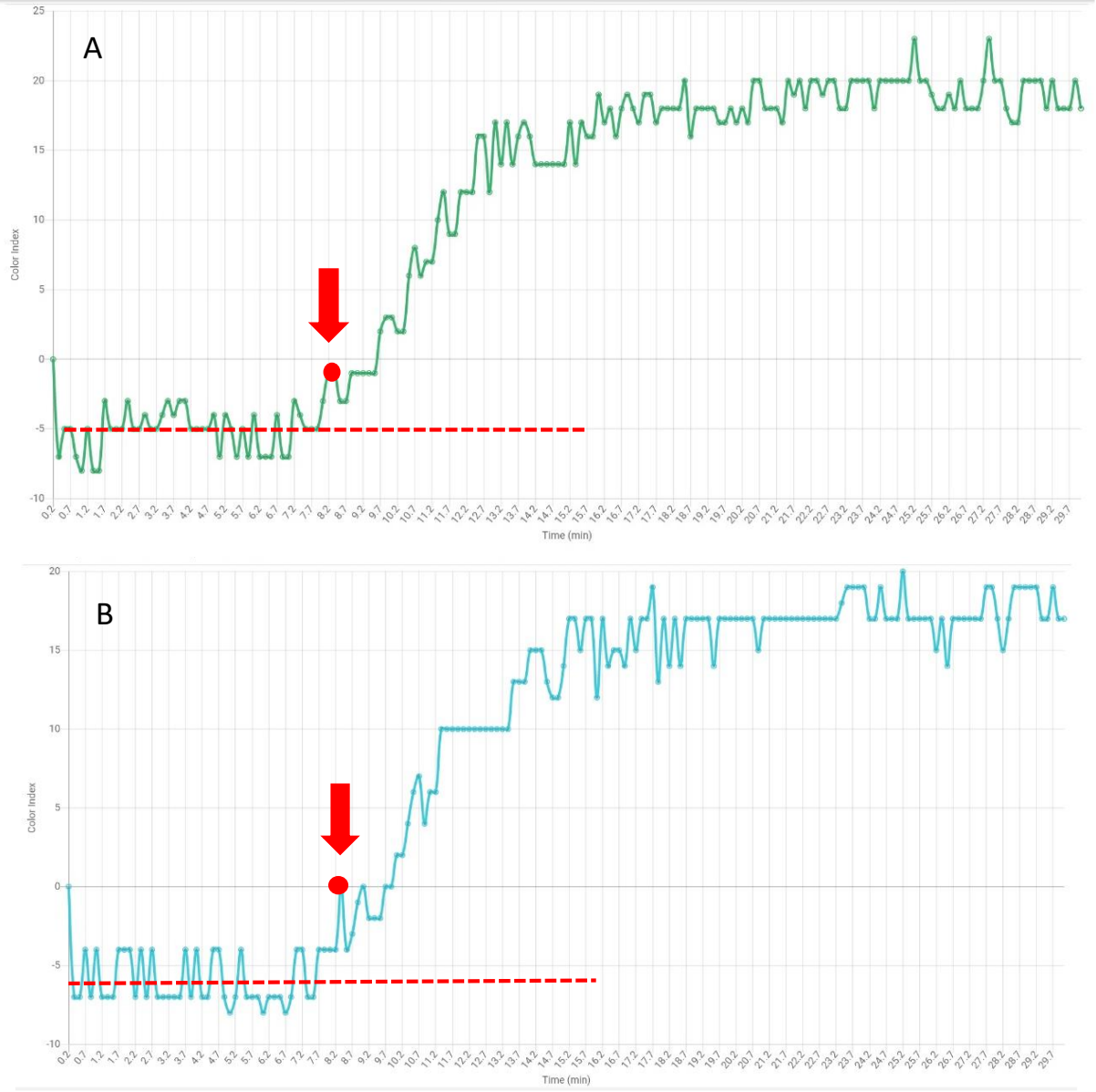


Figure 1: Example of positive results from positive samples A, B.

Negative test results

A negative result is indicated by a flat curve (color index units do not increase) maintained throughout the monitoring period.

When a sample is negative for any target the slope of the curve **WILL NOT CHANGE** (see Figure 2 below).

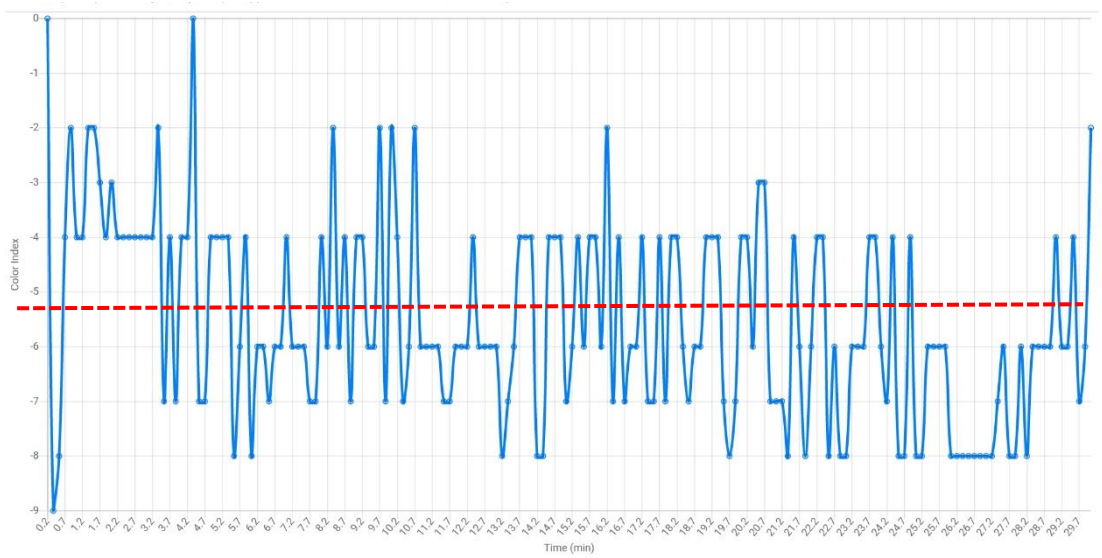


Figure 2: Example of negative result from negative sample.

Invalid test results

A test is considered as invalid if one or more of the following occur:

1. Positive results that appear after **20 minutes** should be considered as **false positive results and should be considered a negative sample**.

2. Negative results using extracted RNA or crude samples directly to the test and the 5X Control Primer mix **ARE NOT** valid and should be repeated. If extracted RNA is used, the RNA isolation process and the preparation of the reactions should be repeated. If a crude sample is used directly to the test, a new specimen should be collected from the patient (recommended after 2-3 days) and tested. In case a new specimen collection is not possible, the preparation of the reactions should be repeated using the initial sample. For more information read the warnings and precautions section.

Limitations of the method

1. For reliable results, it is essential to adhere to the instructions for use of the Flu A qcLAMP kit. Changes in the reaction setup or preparation may lead to the failure of the tests.
2. Results must be interpreted in consideration with all other data gathered for the sample. Interpretation must be performed by personnel trained and experienced with this kind of kit.
3. Mutations within the target sequence may result in failure to detect the virus Influenza A.
4. Inhibitors or other types of interference may give a false-negative result. If this is the case, another sample type or isolation method may be beneficial. Interference studies of the effects of common drugs on colds, on reactions, have not been conducted.
5. This test cannot rule out diseases caused by other viral or bacterial pathogens.
6. Positive and negative predictive values are highly dependent on prevalence. False-negative test results are more likely when the incidence of the disease is high. False-positive test results are more likely when the prevalence of the disease is moderate to low.

7. Tests with Flu A qcLAMP kit can only tell if a person is currently infected with this particular virus. It cannot provide information on other diseases or symptoms and does not tell if a patient has been previously infected with the virus or if patient has any immunity to the virus.
8. A false negative test result may occur if the viral load level in a sample is below the limit of detection of the test or if the sample was improperly collected, handled or transported.

Performance Characteristics

Limit of detection

To evaluate the LOD of the Kit, a LOG10 dilution series of an Influenza A plasmid DNA dissolved in H_2O was measured. The evaluation was performed using one reagent lot and one device.

Analytical Sensitivity, Specificity and Accuracy evaluation

A matrix was created for the under-investigation samples that is identical to a real patient's specimen using nuclease-free water and human genomic DNA.

For the internal evaluation of the performance of the Flu A kit, synthetic DNA sequences of the HA gene of both Influenza A H1N1 and H3N2 were used. The synthetic DNA sequences were reconstituted leading to two initial templates. Afterwards, serial dilutions were performed in order to create positive samples with different concentrations of the target gene.

Blank samples were consisted of nuclease-free water and human genomic DNA to make sure that there are not any non-specific interactions.
Low-level samples were prepared by spiking the Influenza A DNA to achieve a final concentration of 60 copies of the measurand/µl. Low-level samples were aliquoted to contain sufficient quantities to measure. Aliquoted samples were frozen prior to use.
The experimental design consists of replicate measurements on blank (NC) and low level (PC) samples with experiments performed on multiple days on a single instrument.
The accuracy was expressed as the proportion of true positive and true negative in all evaluated cases.

Results of the qualification measurements

The results of the measurements are summarised below:
The Limit of Detection (LOD) was found to be equal to 60 copies/µL.

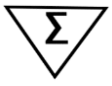
The performance evaluation of the Flu A qcLAMP kit was conducted based on 80 datapoints that were collected. The true positive number is 45, the false positive number is 0, the true negative number is 22, and the false negative is 3. Taking these numbers into consideration, sensitivity (true positive rate), specificity (true negative rate) and accuracy were calculated and sum up below:

Analytical Sensitivity: 97.5%
Analytical Specificity: 100%
Accuracy: 98.7%

Clinical Sensitivity and Specificity evaluation

Clinical tests were not performed.

Used symbols and explanation

Symbols	Explanation
	Website
	E-mail
	Phone number
	*In vitro* diagnostic use
	Batch code
	Catalog number
	Consult electronic instructions for use
	Manufacturer
	European conformity
	Do not use if package is damaged
	Keep dry
	IVD near patient
	Not for self-testing
	Temperature limit (for transportation)
 100 test	Contains sufficient amount for 100 tests
	Serial number
	Use-by-day
	Unique device identifier
	Recyclable

Technical support

For technical support, please contact BIOPIX-T at:

Address: BIOPIX DNA TECHNOLOGY P.C., Science and Technology Park of Crete, N. Plastira 100, Vasilika Vouton, GR-700 13, Heraklion, Greece.
Phone: (+30) 281 0391986
E-mail: support@biopix-t.com / info@biopix-t.com

Literature references

Papadakis et al., "Portable real-time colorimetric LAMP-device for rapid quantitative detection of nucleic acids in crude samples," Scientific Reports volume 12, Article number: 3775 (2022).

Abbreviations

Cat.no: Catalogue number
LAMP: Loop-mediated isothermal amplification
qcLAMP: Quantitative colorimetric loop-mediated isothermal amplification
PCR: Polymerase Chain Reaction
RNA: Ribonucleic Acid
DNA: Deoxyribonucleic Acid
mL: millilitre
µL: microlitre