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The company is registered in the Commercial Register at the Regional Court in Brno under file number C 40647.

EliGene® Influenza A/B/pandemic LC

REF **90058-LC (for 50 samples)**

Kit components:

5 x 150 µl **InfA/H1N1 LC Mix**
5 x 150 µl **InfB LC Mix**
2 x 55 µl Enzyme Mix
1 x 260 µl **IC RNA**
2 x 50 µl **PC Influenza**
1 x **Instruction for Use**

Storage and shelf life after first opening:

All components of the kit must be transported and stored at -20 °C. Kit and remaining MasterMixes must be stored at -20 °C in a dark.

Intended use

EliGene® Influenza A/B/pandemic LC kit is intended for the detection of Influenza A and B RNA together with pandemic variant A/H1N1.

Principle of the method

This diagnostic kit is based on Reverse Transcription-quantitative PCR method in one tube. In this kit primers and labeled probes (FAM, HEX and Cy5) for the detection of Influenza A (H3N2, H1N1, H5N1), Influenza B and for the detection of internal control are used.

Introduction

Influenza is an infectious disease of birds and mammals caused by RNA viruses of the family *Orthomyxoviridae*, the influenza viruses. The most common symptoms are chills, fever, runny nose, sore throat, muscle pains, headache, coughing, weakness and general discomfort. Approximately 33% of people with influenza are asymptomatic. Flu can occasionally lead to pneumonia, either direct viral pneumonia or secondary bacterial pneumonia, even for persons who are usually very healthy. Typically, influenza is transmitted through the air by coughs or sneezes, creating aerosols containing the virus. Influenza can also be transmitted by direct contact with bird droppings or nasal secretions, or through contact with contaminated surfaces. Influenza viruses can be inactivated by sunlight, disinfectants and detergents. Often, new influenza strains appear when an existing flu virus spreads to humans from another animal species, or when an existing human strain picks up new genes from a virus that usually infects birds or pigs. An avian strain named H5N1 raised the concern of a new influenza pandemic after it emerged in Asia in the 1990s, but it has not evolved to a form that spreads easily between people. In April 2009 a novel flu strain evolved that combined genes from human, pig, and bird flu. Initially dubbed "swine flu" and also known as influenza A/H1N1. Influenza B almost exclusively infects humans and is less common than influenza A. This type of influenza mutates at a rate 2–3 times slower than type A.

Primary sample collection, handling and storage

Clinical material:	Recommended RNA isolation procedure:
nasopharyngeal swabs,	Manual: EliGene Viral RNA/DNA FAST Isolation kit (15 min protocol)
swabs, saliva, sputum,	Chemagic Viral DNA/RNA Kit (chemagen-PerkinElmer)
urine	QIAamp Virus Spin Kit or kits recommended by Qiagen

serum, plasma	chemagic Viral DNA/RNA Kit (chemagen-PerkinElmer)
	QIAamp Virus Spin Kit or kits recommended by Qiagen
Automatic isolation:	**ZEPHYRUS Magneto** (ELISABETH PHARMACON)
	MAGNETO BodyFluid DNA/RNA Isolation Kit
	Chemagic 360 Instrument (chemagen-PerkinElmer)
	chemagic Viral DNA/RNA Kit
	chemagic Viral NA/gDNA Kit
	QIAcube Instrument (Qiagen)
	kits recommended by Qiagen

RNA is recommended to be eluted in water for molecular biology. Due to the composition of the elution buffers of some manufacturers, inhibition of PCR reaction by elution buffer compounds may occur. Elution buffer of EliGene Viral RNA/DNA FAST Isolation kit can be used with no fear of PCR inhibition, as well as elution buffers of isolation kits recommended above. If you intend to use isolation kits from other manufacturers, internal control of amplification (RNA) included in this kit must be added to RNA isolation to ensure that inhibition by elution buffer is excluded.

Serum or plasma:

According to standard protocol, take the sample of serum or plasma into sterile tubes.

We recommend use volume 200 µl of serum or plasma and elution volume 50 µl of PCR water. Before the isolation, 5 µl of Internal Control RNA (IC RNA) must be added to the sample after addition of lysis buffer.

Swabs:

These specimens should be collected according to standard protocol in collection tubes.

Recommended swabs:

FLOQSwabs (Copan) – dry swabs or in UTM - Universal Transport Medium (Copan)

Dacron swabs – dry or medium for virus transport MicroTest™ M4RT or MicroTest™ M6 (Thermo Scientific)

Other polymer fiber collection kits – dry or with transport medium for viruses.

Do not use cotton swabs that can inhibit PCR.

Specimens should be transported to the laboratory at 4 °C (blue ice). They are stable minimally 72 hours from sampling at 4°C. In the case, that you have no possibility to transport dry swabs to laboratory at 4°C, at room temperature dry swabs should be transported until 6 hours.

For storage of samples longer than 72 hours, freeze sample to -20 °C.

Dry swabs should be submerged in lysis buffer according to instruction manual of used isolation kit. After swab removal, **5µl of Internal Control RNA (IC RNA) must be added to the sample used for RNA isolation after addition of lysis buffer.**

In the case of sampling in transport medium, 200 µl or quantity recommended by instruction manual of used isolation kit should be used for RNA isolation. 5µl of Internal Control RNA (IC RNA) must be added to the sample used for RNA isolation after addition of lysis buffer.

Additional required equipment

- Automatic pipettes 1-1000 µl and sterile tips with filter DNA-, RNA- free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for RealTime PCR – the kit is designed for RealTime Systems LightCycler® 480, QuantStudio 5 Real-Time PCR Systems (ThermoFisher Scientific), Rotor-Gene Q (Qiagen) and Real-Time PCR system CFX96 (Bio-Rad). The RT-qPCR for the detection of influenza RNA utilizes TaqMan technology (FAM and HEX probes) and can be performed on other instruments that can work in FAM and HEX channels
- Lab safety gloves

Configuration of Real Time instrument

- For Influenza A and B detection the probe labelled with FAM is used (exc. 494 nm – em. 518 nm)
- For Influenza A/H1N1 detection the probe labelled with Cy5 is used (exc. 618 nm – em. 660 nm)
- For Internal control the probe labelled with HEX is used (exc. 520 nm – em. 548 nm)

LightCycler® 480 (Roche):

Before the using of EliGene® Influenza A/B/pandemic LC kit it is necessary to perform spectral calibration with EliGene® LC Spectral Calibration Kit 2 (Cat. No. 90099-SC2)! Once the spectral calibration was done; it is valid until the instrument is upgrading or optics is changed.

Please, use white plates only intended for LightCycler® 480. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates.

In option Detection format choose “3 Color Hydrolysis probe”.

Set up the following temperature profile:

Step 1 - Analysis mode “None”, 1 Cycle

55°C	15 min	Ramp rate (4.4°C/s)	Acquisition mode “None”

Step 2 - Analysis mode “None”, 1 Cycle

95°C	2 min	Ramp rate (4.4°C/s)	Acquisition mode “None”

Step 2 - Analysis mode “Quantification”, 45 Cycles

95°C	5 s	Ramp rate (4.4°C/s)	Acquisition mode “None”
55°C	15 s	Ramp rate (2.2°C/s)	Acquisition mode “Single”
67°C	15 s	Ramp rate (4.4°C/s)	Acquisition mode “None”

Step 3 - Analysis mode “None”, 1 Cycle

40°C	20 s	Ramp rate (2.2°C/s)	Acquisition mode “None”

The complete temperature profile can be up-loaded from Run Template “EliGene INFLUENZA LC_LC480_v02.ixo”. The Run Template can be imported to the software in menu “Navigator” by clicking to icon “Import” from the CD included in the kit.

QuantStudio 5 Real-Time PCR Systems (ThermoFisher Scientific):

Use the Experiment type," Presence/Absence", Chemistry "TaqMan Probes", and Run Mode "Standard". As a reporter dyes use FAM (InfA, InfB), VIC (IC RNA), Cy5 (InfA H5N1**). Do not use any passive reference dye!**

Set up the following temperature profile:

Holding stage

55°C 15 min Ramp rate (1.6°C/s)

Holding stage

95°C 2 min Ramp rate (1.6°C/s)

Cycling stage – 45 cycles

95°C 5 s Ramp rate (1.6°C/s)

55°C 15 s Ramp rate (1.6°C/s) Data collection ON

67°C 15 s Ramp rate (1.6°C/s)

Post-Read Stage

40°C 20 s Ramp rate (1.6°C/s)

Collect emission signal at the second step at 55 °C

The complete temperature profile can be up-loaded from Run Template "EliGene INFLUENZA LC_QS5_v00.edt". The Run Template can be copied from the CD included in the kit.

RotorGene 6000 or Q (Qiagen):

In the "New Run" window choose "Three Step" run

Choose the appropriate "Rotor Type" and click "Next".

Set up the following temperature profile:

Holding stage

55°C 15 min

Holding stage

95°C 2 min

Cycling stage – 45 cycles

95°C 5 s

55°C 15 s Acquiring in channels "Green", "Yellow" and "Red"

67°C 15 s

Holding stage

40°C 20 s

For the Gain optimization in all channels select option "Automatic gain optimization before first acquisition". The complete temperature profile can be up-loaded from Run Template "EliGene INFLUENZA LC_Q-GENE_v00.ret". The Run Template can be copied from the CD included in the kit.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Startup Wizard Create a new Experiment for CFX96 instrument and Create New Protocol.

Set up the following temperature profile:

Step 1 55°C 15 min

Step 2 95°C 2 min

Step 3	95°C	5 s
Step 4	55°C	15 s + Plate Read
Step 5	67°C	15 s
Step 6	*GOTO Step 3*	*44x*
Step 7	40°C	20 s

Enter the Sample Volume 20ul

Collect emission signal at the Step 4 at 55° C.

For filter settings, use the "Scan Mode" All Channels, and in Plate Manager, select for the samples only fluorophores FAM, HEX, and Cy5. Then assign the samples with positions and Targets as an Unknown sample (Samples) or Standard. The complete temperature profile can be up-loaded from Run Template "EliGene INFLUENZA LC_CFX96_v00.prcd". The Run Template can be copied from the CD included in the kit.

Real-Time PCR system iCycler iQ5 (Bio-Rad)

In window "Protocol" chooses "Create New"and set-up following temperature profile:

Cycle 1 - 1 Repeat

55°C	15 min	

Cycle 2 - 1 Repeat

95°C	2 min	

Cycle 3 – 45 Repeats

95°C	5 s	
55°C	15 s	Acquisition of data "Real Time"
67°C	15 s	

Cycle 4 - 1 Repeat

40° C	1 min	

In window "Plate" chooses "Create New" and then select menu "Select/Add Fluorophores" and choose dyes FAM, HEX and Cy5. Thereafter enter individual samples and start run.

Reagent preparation

- To avoid the contamination, keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, briefly mixed on vortex and shortly spun.
- Add 5 µl of Internal Control RNA (IC RNA) to sample with lysis buffer. Never add Internal Control RNA to isolated RNA before starting PCR!
- In case of using iCycler iQ5 instrument it is necessary to add fluorescein standard in a final concentration of 10 nM to correct normalisation and evaluation of measured data. It is possible to use 100 x EliFluorescein Standard when you add to the tube with InfA/H1N1 and InfB mix 2 ul of 100 x EliFluorescein Standard.

Preparation of Reaction Mix

1. Take the InfA/H1N1 and InfB Mix tubes and the Enzyme Mix tube and thaw at room temperature. Immediately after thawing, spin briefly in centrifuge. Prepare the InfA/H1N1 and InfB Master Mixes by mixing 14 µl of InfA/H1N1 and InfB mixes with 1 µl of Enzyme Mix per reaction and spin

briefly.

2. Detection: Add 15 µl of the InfA/H1N1 and InfB Master Mixes to the amplification tubes or plates and add 5 µl of the isolated RNA sample. Be careful when pipetting the sample to avoid cross-contamination of the samples. The prepared Master Mix should be used within 30 minutes and cannot be reused. Do not freeze prepared Master Mix.
3. Positive Control: Pipette 15 µl of the InfA/H1N1 and InfB Master Mixes separately into the amplification tube or plate. Then add 5µl of PC Influenza. Be careful when pipetting the positive control to avoid contamination of samples. **Use a different micropipette for pipetting only positive controls.**

Insert the microtubes or plate into the RealTime PCR instrument and run the program as described in Configuring the RealTime PCR Instrument above.

Result viewing

LightCycler® 480 (Roche):

In “Sample Editor” menu choose “Abs Quant” workflow.

In menu “Analysis” choose “Abs Quant/2nd Derivative Max” option.

In Analysis window click to “Color Comp” icon and choose calibration for EliGene® Influenza A/B/pandemic LC kit. Analyze results by clicking to icon “Calculate”.

Positive result for Influenza A: The positive result is characterized by amplification and growth of signal in FAM channel (465-510) of InfA/H1N1 LC Mix reaction. In a case of negative results the amplification will not occur.

Positive result for Influenza A/H1N1: The positive result is characterized by amplification and growth of signal in Cy5 channel (618-660) of InfA/H1N1 LC Mix reaction. In a case of negative results the amplification will not occur.

Positive result for Influenza B: The positive result is characterized by amplification and growth of signal in FAM channel (465-510) of InfB LC Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (533-580) in both InfA/H1N1 LC and InfB LC Mix.

The values of Concentration correspond to the quantity of positive result; “Negative” means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

QuantStudio 5 Real-Time PCR Systems (ThermoFisher Scientific):

In “Analyse Settings” choose “Automatic Treshold” and “Automatic Baseline” option and analyze results.

Positive result for Influenza A: The positive result is characterized by amplification and growth of signal in FAM channel of InfA/H1N1 LC Mix reaction. In a case of negative results the amplification will not occur.

Positive result for Influenza A/H1N1: The positive result is characterized by amplification and growth of signal in Cy5 channel of InfA/H1N1 LC Mix reaction. In a case of negative results the amplification will not occur.

Positive result for Influenza B: The positive result is characterized by amplification and growth of signal in FAM channel of InfB LC Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel in both InfA/H1N1 LC and InfB LC Mix.

Rotor-Gene Q (Qiagen):

Click to "Analysis" icon in the menu and choose the Analysis option "Quantitation". In the "Quantitation

Analysis" window, select "Dynamic Tube" and "Slope Correct" option. In the option "Outlier Removal" setup NTC Threshold value for 10 %.

Positive result for Influenza A: The positive result is characterized by amplification and growth of signal in FAM channel (Green) of InfA/H1N1 LC Mix reaction. In a case of negative results the amplification will not occur.

Positive result for Influenza A/H1N1: The positive result is characterized by amplification and growth of signal in Cy5 channel (Red) of InfA/H1N1 LC Mix reaction. In a case of negative results the amplification will not occur.

Positive result for Influenza B: The positive result is characterized by amplification and growth of signal in FAM channel (Green) of InfB LC Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (Yellow) in both InfA/H1N1 LC and InfB LC Mix.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Data Analysis window choose “Quantification”. In “Settings” menu choose option “Baseline Threshold” and select “Baseline Cycles” option as “Auto Calculated” and Single “Threshold” option as “Auto Calculated”.

In Data Analysis window select a single fluorophore (FAM, HEX or Cy5) by the clicking the box next to the fluorophore name located under the amplification chart and read the results for individual samples.

Positive result for Influenza A: The positive result is characterized by amplification and growth of signal in FAM channel of InfA/H1N1 LC Mix reaction. In a case of negative results the amplification will not occur.

Positive result for Influenza A/H1N1: The positive result is characterized by amplification and growth of signal in Cy5 channel of InfA/H1N1 LC Mix reaction. In a case of negative results the amplification will not occur.

Positive result for Influenza B: The positive result is characterized by amplification and growth of signal in FAM channel of InfB LC Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (Yellow) in both InfA/H1N1 LC and InfB LC Mix.

Real-Time PCR system iCycler iQ5 (Bio-Rad)

In window “Data File“ chooses “Analyze“ and in menu „Analysis Mode“ chooses “PCR Base Line Substracted Curve Fit“. Thereafter in menu „Base Line Treshold Parameter“ consequently chooses for all analysed dyes (FAM, HEX a Cy5) in parameter „Crossing Treshold“ possibility “User Defined“ and enter the value of Treshold Position of 100.

Positive result for Influenza A: The positive result is characterized by amplification and growth of signal in FAM channel of InfA/H1N1 LC Mix reaction. In a case of negative results the amplification will not occur.

Positive result for Influenza A/H1N1: The positive result is characterized by amplification and growth of signal in Cy5 channel of InfA/H1N1 LC Mix reaction. In a case of negative results the amplification will not occur.

Positive result for Influenza B: The positive result is characterized by amplification and growth of signal in FAM channel of InfB LC Mix reaction. In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel in both InfA/H1N1 LC and InfB LC Mix.

Interpretation of results

Negative result:

If the increasing of amplification signal in FAM and Cy5 channel of InfA/H1N1 LC Mix does not appear before cycle number 40, the result of test should be interpreted as probably Influenza A and A/H1N1 negative or with concentration of RNA below the detection limit of this kit (15 genomic RNA/reaction). If the increasing of amplification signal in FAM channel of InfB LC Mix does not appear before cycle number 40, the result of test

should be interpreted as probably Influenza B negative or with concentration of RNA below the detection limit of this kit (15 genomic RNA/reaction).The signal for Internal Control must be positive – see article Quality control. This result does not exclude the occurrence of Influenza A, A/H1N1 or B infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed Influenza RNA.

Positive result:

Amplification signal in FAM channel of InfA/H1N1 LC Mix appears before cycle number 40. Influenza A RNA was detected in the sample. The sample is Influenza A RNA positive.
Amplification signal in Cy5 channel of InfA/H1N1 LC Mix appears before cycle number 40. Influenza A/H1N1 RNA was detected in the sample. The sample is Influenza A/H1N1 RNA positive.
Amplification signal in FAM channel of InfB LC Mix appears before cycle number 40. Influenza B RNA was detected in the sample. The sample is Influenza B RNA positive.

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of RNA analyses.

Inhibited sample:

In the case that increasing of amplification signal specific for Influenza A, Influenza A/H1N1 in FAM and Cy5 channels of InfA/H1N1 LC Mix and also increasing of amplification signal specific for internal control in HEX channel is not observed, it is necessary to repeat the analysis. The best, there is to use samples prepared by new RNA isolation.
In the case that increasing of amplification signal specific for Influenza B in FAM channel of InfB LC Mix and also increasing of amplification signal specific for internal control in HEX channel is not observed, it is necessary to repeat the analysis. Make sure the elution buffer does not inhibit the PCR reaction. In this case, it is recommended to perform elution into the water for molecular biology.

Control procedure

EliGene® Influenza A/B/pandemic LC kit involves Internal Control. Internal Control follows the quality of RNA isolation and detects the inhibition of reverse transcription and amplification. The internal control must be added directly to the sample with lysis buffer before the viral RNA isolation. Suppose the sample is RNA negative for the specific amplification in FAM and Cy5 channels. In that case, there must be present an amplification in the HEX channel (internal amplification control) with Cq value lower than 35.

Reference material:

To monitor the all examination process covering RNA isolation and RealTime PCR detection is possible to use reference viral material positive for given Influenza starin. The commercial positive material is not available.

Troubleshooting:

1. If there is no amplification of Internal Control, there is some problem in the isolation of RNA or the kit is after the expiration date or there is RealTime instrument breakdown.
2. If there is no amplification of Positive Control, the kit is after the expiration date or there is RealTime instrument breakdown.

Performance characteristics

Analytical performance characteristics:

EliGene® Influenza A/B/pandemic LC kit is 5 genomic RNA (Influenza A, A/H1N1, B) in reaction mix. The sensitivity of the method was verified as follows. The samples with known concentrations of Influenza A, A /

H1N1 and B genomic RNAs were used. The test was performed a total of three times. Detection of influenza was 100% successful in all samples, at least 5 genomic RNAs in the reaction mix.

Analytical sensitivity is 5 copies of Influenza virus RNA in reaction Mix.

Analytical specificity of method is 100%. Analytical specificity of method was analyzed by comparison of primers and probes with all known RNA and DNA sequences in GenBank database. Analytical specificity was also analyzed by the addition of DNA/RNA from SARS-Cov2, EBV, HSV1, HSV2, VZV, Enterovirus, MTB, Borrelia spp., *C. trachomatis, E. coli, A. niger, C. albicans* and RNA from HCV, RSV1, RSV2 to the reaction Mix. These DNA or RNA did not give false positive result for Influenza.

Clinical specificity was tested on 100 clinical specimens. EliGene® Influenza A/B/pandemic LC kit showed 100% match in detection of *Influenza A* (10 from 10 specimens), *Influenza A/H1N1* (5 from 5 specimens) and *Influenza B (*8 from 8 specimens) with results determined by CE-IVD reference kit.

Diagnostic performance characteristics:

Measuring interval

The kit enables the detection of 10^1 - 10^8 of viral RNA molecules in reaction Mix.

Internal control of quality

As an internal control of quality the Internal Control for checking the process of RNA isolation together with Positive Control for functional control of MasterMix and as a reference sample is used.

Limitation of the examination procedure

The sensitivity of kit depends on handling with specimen (isolation of RNA). It is strictly recommended to use isolation kits and procedures mentioned above.

Negative result does not exclude the occurrence of Influenza infection. Results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed Influenza RNA. The presence of Influenza RNA of infected persons is dependent on infection phase.

Biological reference intervals

Not applicable information for this kit.

Warning

After mixing MasterMix is stable for 2 weeks at -20 °C. Do not freeze tubes with MasterMix repeatedly! Do not mix components of the kits of different lots.

Warnings and general precautions

This kit is intended for *in vitro* use only.

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.

- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer´s kit.

Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

The tubes containing InfA/H1N1 LC Mix and InfB LC Mix are disposable and therefore must be used once only in the preparation of the reaction mixture.

These Mixes carry the following safety warnings (S):

S36/37. Wear suitable protective clothing and gloves

The tubes containing IC RNA are disposable and therefore must be used once only in the preparation of the reaction mixture.

Literature

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Symbols

REF	Catalog number
	Upper limit of temperature
LOT	Batch code
	Use by (last day of month).
IVD	*In vitro* diagnostic medical device
CE	Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.
	Contains sufficient for "N" tests
	Attention, consult instructions for use.
	Manufacturer

Manufacturer

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