



For Professional Use Only

eSens Influenza virus A/H5N1 QL PCR kit

REF ES3301B

Instructions for Use

1 INTENDED USE

eSens Influenza virus A/H5N1 QL PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Influenza virus A* RNA and identifying of H5N1 subtype in the biological material (nasal and oropharyngeal swabs or washes; tracheal aspirate; feces; autopsy material; animal material (bird droppings, cloak, pharyngeal, tracheal swabs, tracheal wash samples, visceral organs from fallen animals)) using real-time hybridization-fluorescence detection of amplified products.

NOTE: The results of PCR analysis are taken into account in complex diagnostics of disease.

2 PRINCIPLE OF PCR DETECTION

Influenza virus A/H5N1 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *Influenza virus A/H5N1* primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

eSens Influenza virus A/H5N1 QL PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-rec (IC)). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

eSens Influenza virus A/H5N1 QL PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP). The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTP is a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target

amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE
PCR-mix-1-FRT (SC) Influenza virus A		
cDNA-target	<i>Influenza virus A</i> cDNA	Internal Control cDNA
Target gene	M gene encoding matrix protein	Artificially synthesized sequence
PCR-mix-1-FRT (SC) Influenza virus A H5N1		
cDNA-target	<i>Influenza virus A</i> H5 cDNA	<i>Influenza virus A</i> N1 cDNA
Target gene	HA gene encoding hemagglutinin type 5	NA gene encoding neuraminidase type 1

3 CONTENT

eSens Influenza virus A/H5N1 QL PCR kit (ES3301B) includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT (SC) Influenza virus A	colorless clear liquid	0.12	5 tubes
PCR-buffer-Flu	colorless clear liquid	0.28	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.06	1 tube
Positive Control cDNA Influenza virus A (C+A)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.6	3 tubes
Internal Control STI-rec (IC)**	colorless clear liquid	0.12	5 tubes

* must be used in the extraction procedure as Negative Control of Extraction.

** add **10 µl** of **Internal Control STI-rec (IC)** during the RNA extraction procedure directly to the sample/lysis mixture.

Reagents for identifying *Influenza virus A H5N1* subtype:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT (SC) <i>Influenza virus A H5N1</i>	colorless clear liquid	0.12	5 tubes
Positive Control cDNA <i>Influenza virus A H5 (C_{H5})</i>	colorless clear liquid	0.1	1 tube
Positive Control cDNA <i>Influenza virus A N1 (C_{N1})</i>	colorless clear liquid	0.1	1 tube

eSens Influenza virus A/H5N1 QL PCR kit is intended for 55 reactions (including controls).

4 ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Transport medium.
- 0.9 % saline solution or phosphate buffer.
- Reagent for pretreatment of viscous fluids (sputum, aspirates).

For RNA extraction, reverse transcription and amplification

- **RNA extraction kit (REVERTA-L)**
- Disposable tightly closed polypropylene 1.5- ml tubes for sampling and pretreatment.
- Thermostat with working temperature 25 °C to 100 °C (suitable for Eppendorf tubes).
- Vacuum aspirator with flask for removing supernatant.
- Reverse transcription kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile RNase-free pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene microtubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5 GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.

- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

 Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6 SAMPLING AND HANDLING

NOTE: Sampling, transportation and pretreatment of biological and autopsy material must be carried out in strict adherence to the local authorities' requirements regarding to the organization and carrying out of laboratory diagnostics of human diseases caused by highly virulent strains of *Influenza* type A viruses.

eSens Influenza virus A/H5N1 QL PCR kit is intended for analysis of the RNA extracted with RNA extraction kits from the biological material (nasal and oropharyngeal swabs or washes, tracheal aspirate, feces, and autopsy material, animal material (bird droppings, cloak, pharyngeal, tracheal swabs, tracheal wash samples, visceral organs from fallen animals).

Sampling

Human material:

6.1 Nasal swab samples.

Nasal swab samples are obtained using sterile dry floccated swabs with plastic shafts for nasopharyngeal swabs. Gently insert the swab along the external nasal wall to a depth of 2–3 cm towards the inferior nasal concha. Then move the swab slightly lower, insert it in the inferior nasal meatus under the inferior nasal concha, rotate, and remove along the external nasal wall.

When the material is obtained, insert the swab into a sterile disposable tube with 500 µl of transport medium. Break off the end of shaft to allow tight closing of the tube cap. Close the tube with the solution and the swab.

6.2 Oropharyngeal swab samples.

Oropharyngeal swab samples are obtained using sterile dry rayon swabs with plastic shafts for oropharyngeal swabs. Rotate the swab over the surface of tonsils, palatine arches, and posterior wall of pharynx after gargling the oral cavity with water. When material is obtained, insert the swab into a sterile disposable tube with 500 µl of transport medium. Break off the end of shaft to allow tight closing of tube cap. Close the tube with the solution and the swab.

NOTE: It is recommended to combine nasal and oropharyngeal swabs in a single tube.

For this purpose, place the ends of both shafts into one tube containing 500 µl of transport medium and analyze them as a single sample.

6.3 Nasal wash.

Patient should sit with head tilted backward. Instill 3-5 ml of warm sterile saline solution into each nostril using disposable probe or syringe. Collect the sample from both nostrils in a single sterile tube using funnel. Only an autoclaved funnel should be used.

6.4 Oropharyngeal wash.

It is necessary to rinse the mouth with water before sampling. After that rinse the throat thoroughly with 8–10 ml of saline for 10–15 s. Collect the sample to a sterile tube using a funnel. Only an autoclaved funnel should be used.

6.5 Fecal sample.

Fecal sample (1.0 – 3.0 g) should be obtained from a sterile disinfected bedpan or a chamber-pot and transferred to a sterile container with a disposable spatula.

6.6 Autopsy sample.

Autopsy sample should be immediately placed in a sterile disposable container and frozen otherwise it should be examined within 1 hour from the time of sample collection. Store the samples at minus 68 °C for 1 year. Only one freeze-thaw cycle of clinical material is allowed.

Animal material:

Samples obtained from birds

6.7 Droppings.

Droppings (4.0 – 5.0 g) are collected to a sterile container.

6.8 Cloak, pharyngeal, tracheal swabs.

Cloak, pharyngeal, tracheal swabs are obtained using a sterile dry rayon swabs with plastic shafts. The swab is placed in a sterile disposable tube containing 500 µl of respiratory transport medium, sterile saline or phosphate buffer (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM sodium monophosphate, 2mM potassium diphosphate; pH 7.5±0.2. Store phosphate buffer at 2–8 °C for 1 year in a tightly sealed polypropylene tube). Break off the end of shaft or cut it off to allow tight closing of the tube cup. Close tube with solution and the swab.

6.9 Tracheal wash sample.

Tracheal wash sample is obtained using a sterile saline.

Samples obtained from other fallen animals

6.10 Visceral organs (fragments of trachea and lungs).

Visceral organs (fragments of trachea and lungs) are collected to sterile disposable containers.

Store the above-mentioned material at 2-8 °C for 1 day before the test or at the temperature below minus 16°C for one week.

Pretreatment

6.11 Swabs and washes.

Swabs and washes are used without pretreatment.

6.12 Tracheal aspirate.

Mucolysin reagent (180-CE) is additionally required. Treat samples according to manufacturer's instructions. The prepared solution (50 µl) is used for RNA extraction. The remaining sample can be frozen for further use.

6.13 Autopsy material and visceral organs of animals.

Autopsy material and visceral organs of animals should be homogenized using a sterile porcelain mortar and pestle. Then prepare the 10 % suspension in sterile saline or phosphate buffer. Transfer the suspension to a 1.5-ml tube and spin at 10,000 rpm for 30 s. Use the supernatant for RNA extraction.

6.14 Human feces.

Prepare fecal suspension from native feces that were not frozen.

Preparation of 10-20% fecal suspension (can be omitted for watery feces).

Take the required number of 1.5-ml tubes. Pipette 0.8 ml of phosphate buffer or sterile saline into each tube. Transfer 0.1 g (0.1 ml) of fecal sample to the tube using a disposable spatula and stir well on vortex to obtain a homogenous suspension.

If the material cannot be studied within 1 day and/or if continuous storage is required, add glycerol (final concentration, 10–15 %) to 10–20 % fecal suspension. Thoroughly homogenize samples with glycerol, incubate for 30–40 min, and then freeze.

Preparation of clarified fecal extract

Vortex the tubes with the prepared suspension (freshly made or frozen with glycerol) or liquid feces, then centrifuge at 10,000 g (12,000 rpm) for 5 min. Use the supernatant for RNA extraction.

6.15 Bird droppings.

Use 4.0–5.0 g of droppings for analysis. Prepare a 10 % suspension in sterile saline, thoroughly resuspend, and decant for 10 min. Transfer the supernatant to the tube and centrifuge it at 12,000 rpm for 5 min. Use the supernatant for RNA extraction.

7 WORKING CONDITIONS

eSens Influenza virus A H5N1 QL PCR kit should be used at 18–25 °C

8 PROTOCOL

8.1 RNA extraction

Any commercial nucleic acid extraction kit, if IVD-CE validated for the indicated specimen types, could be used.

Ecoli Dx, s.r.o. recommends:

- For the manual extraction
 - **RIBO-prep** (K2-9-Et-100-CE)
- For the automatic extraction
 - **ePure Viral Nucleic Acid Extraction Kit** (E2003)

NOTE: Extract the RNA according to the manufacturer's protocol.
The DNA extraction for each sample is carried out in the presence of **Internal Control-F (IC)**.

8.2 Reverse transcription

It is recommended to use the following kit for the complementary DNA (cDNA) synthesis from the RNA:

- **REVERTA-L** (K3-4-50-CE); (K3-4-100-CE)

NOTE: Carry out the reverse transcription according to the manufacturer's protocols.

8.3 Preparing PCR

8.3.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

The total reaction volume is **25 µl**, the volume of cDNA sample is **10 µl**.

8.3.1.1 Detection of *Influenza virus A* RNA

1. Prepare required number of the tubes with **PCR-mix-1-FRT (SC) Influenza virus A** (one tube is intended for 11 reactions). Vortex the tube, then centrifuge shortly.
2. For N reactions (including 2 controls), add to a new tube:

10*(N+1) µl of **PCR-mix-1-FRT (SC) Influenza virus A**,
5.0*(N+1) µl of **PCR-buffer-Flu**, and
0.5*(N+1) µl of **polymerase (TaqF)**.

Vortex the tube, then centrifuge shortly. Transfer **15 µl** of the prepared mixture to each tube.

3. Using tips with aerosol filter, add **10 µl** of **cDNA samples** obtained at the RNA reverse transcription stage.
4. Carry out the control amplification reactions:

NCA	-	Add 10 µl of TE-buffer to the tube labeled NCA (Negative Control of Amplification).
C+A	-	Add 10 µl of Positive Control cDNA Influenza virus A (C+A) to the tube labeled C+A (Positive Control of Amplification).
C-	-	Add 10 µl of cDNA samples obtained by extraction and reverse transcription of the Negative Control (C-) reagent to the tube labeled C- (Negative control of Extraction).

8.3.1.2 Identifying *Influenza virus A* H5N1 subtype

NOTE: cDNA samples with positive results after detection of *Influenza virus A* RNA are used for identifying of *Influenza virus A* H5N1 subtype.

1. Prepare required number of the tubes with **PCR-mix-1-FRT (SC) Influenza virus A H5N1** (one tube is intended for 11 reactions). Vortex the tube, then centrifuge shortly.
2. For N reactions (including 2 controls) add to a new tube:

10*(N+1) µl of **PCR-mix-1-FRT (SC) Influenza virus A H5N1**,
5.0*(N+1) µl of **PCR-buffer-Flu** and
0.5*(N+1) µl of **polymerase (TaqF)**.

Vortex the tube, then centrifuge it briefly. Transfer **15 µl** of the prepared mixture to each tube.

- Using tips with aerosol filter, add **10 µl** of **cDNA samples** obtained at the RNA reverse transcription stage.
- Carry out the control amplification reactions:

NCA	-	Add 10 µl of TE-buffer to the tube labeled NCA (Negative Control of Amplification).
C+H5	-	Add 10 µl of Positive Control cDNA Influenza virus A H5 to the tube labeled C+H5 (Positive Control of Amplification).
C+N1	-	Add 10 µl of Positive Control cDNA Influenza virus A N1 to the tube labeled C+N1 (Positive Control of Amplification).
C-	-	Add 10 µl of cDNA samples obtained by extraction and reverse transcription of the Negative Control (C-) reagent to the tube labeled C- (Negative control of Extraction).

8.3.2 Amplification

- Create a temperature profile on your instrument as follows:

Table 2

eSens-1 amplification program

Step	Rotor-type Instruments (e.g Rotor-Gene Q or equivalent)			Plate-type Instruments (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent)		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	95	15 min	1	95	900 s	1
2	95	10 s	10	95	15 s	42
	54	20 s				
	72	10 s				
3	95	10 s	35	54	25 s Fluorescent signal detection	
	54	20 s Fluorescent signal detection				
	72	10 s			25 s	

Fluorescent signal is detected in the channels for **FAM** and **JOE** fluorophores.

- Adjust the fluorescence channel sensitivity.
- Insert tubes into the reaction module of the device.
- Run the amplification program with fluorescence detection.
- Analyze results after the amplification program is completed.

8.4 Instrument Settings

Test settings for rotor-type instruments

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
Sample	<i>Influenza virus A</i>				
FAM/Green	from 10FI to 20FI	0.1	On	On	10 %
JOE/Yellow	from 5FI to 10FI	0.1	On	On	20 %
Sample	<i>Influenza virus A H5 N1</i>				
FAM/Green	from 5FI to 10FI	0.1	On	On	10 %
JOE/Yellow	from 5FI to 10FI	0.1	On	On	10 %

Test settings for plate-type instruments

Channel	Threshold
FAM, HEX	The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

9 DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

In case of detection of *Influenza virus A* RNA:

- The signal of the *Influenza virus A* cDNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the IC cDNA amplification product is detected in the channel for the JOE fluorophore.

In case of identifying of *Influenza virus A* H5 N1 subtype:

- The signal of the *Influenza virus A* H5 cDNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *Influenza virus A* N1 cDNA amplification product is detected in the channel for the JOE fluorophore.

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a C_t value of the cDNA sample in the corresponding column of the results grid.

Principle of interpretation is the following:

Detection of *Influenza virus A* RNA

1. *Influenza virus A* cDNA is detected if the C_t value determined in the results grid in the channel for the FAM fluorophore is less than the specified boundary C_t value.
2. *Influenza virus A* cDNA is not detected in a sample if the C_t value is not determined (absent) in the channel for FAM fluorophore or greater than the specified boundary C_t value, whereas the C_t value determined in the channel for the JOE fluorophore is less than the specified boundary C_t value.

- The result is invalid if the *Ct* value is not determined (absent) in the channel for FAM fluorophore, whereas the *Ct* value in the channel for the JOE fluorophore is not determined (absent) or greater than the specified boundary *Ct* value. In such cases, the PCR analysis should be repeated starting from the RNA extraction stage.

Identifying of *Influenza virus A H5N1* subtype

- Influenza virus A H5* cDNA is identified in a sample if the *Ct* value determined in the channel for the FAM fluorophore is less than the specified boundary *Ct* value.
- Influenza virus A N1* cDNA is identified in a sample if the *Ct* value determined in the channel for the JOE fluorophore is less than the specified boundary *Ct* value
- The required H5N1 subtype of *Influenza virus* is not identified in a sample if the *Ct* value is not determined (absent) in the channel for the FAM and/or JOE fluorophore, whereas the *Ct* value in the channel for the JOE fluorophore (IC detection) is less than the specified boundary *Ct* value.
- If the positive signals are determined simultaneously in the sample in the channels for the FAM and JOE fluorophores this indicates that *Influenza virus A H5N1* is present in the sample, or several *Influenza virus* subtypes with haemagglutinin 5 and neuraminidase 1 are present simultaneously.

The result is **equivocal** for the presence of the pathogen or the target gene, if the *Ct* value determined in the channel for detection of the pathogen or the target-gene is less than the specified boundary *Ct* value. In such cases, the PCR should be repeated. If the same result is obtained, the sample is considered positive for this target-gene.

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 3 - Table 6).

Table 3

Boundary *Ct* values for control and test samples (detection of *Influenza virus A* cDNA.)

Sample	FAM/Green channel	JOE/Yellow channel
C-	Absent	31
NCA	Absent	Absent
C ⁺ _A	33	Absent
Test samples	33	31

Table 4

Boundary Ct values for control and test samples (Identification of the *Influenza virus A H5N1* subtype)

Sample	FAM/Green channel	JOE/Yellow channel
C-	Absent	Absent
NCA	Absent	Absent
C _{+NI}	33	Absent
C _{+H5}	Absent	33
Test samples	33	33

Table 5

Boundary Ct values for control and test samples (detection of *Influenza virus RNA*)

Sample	FAM channel	HEX channel
C-	Absent	31
NCA	Absent	Absent
C _{+A}	33	Absent
Test samples	33	31

Table 6

Boundary Ct values for control and test samples (Identifying of *Influenza virus A H5N1* subtype)

Sample	FAM channel	HEX channel
C-	Absent	Absent
NCA	Absent	Absent
C _{+NI}	33	Absent
C _{+H5}	Absent	33
Test samples	33	33

10 TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. The Ct value determined for the Positive Control of amplification (C+) in the channels for the respective fluorophores is greater than the boundary Ct value or absent. The amplification should be repeated for all the samples in which the specific RNA was not detected.
2. The Ct value determined for the Negative Control of Extraction (C-) and/or Negative Control of Amplification (NCA) in the channel for the pathogen detection is less than the specified boundary Ct value. The PCR analysis (beginning with the RNA extraction stage) should be

repeated for all samples in which specific RNA was detected to exclude the probable contamination.

11 TRANSPORTATION

eSens Influenza virus A/H5N1 QL PCR kit should be transported at 2–8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens Influenza virus A/H5N1 QL PCR kit** are to be stored at 2–8 °C when not in use (except for Polymerase (TaqF), PCR-mix-1-FRT (SC) *Influenza virus A* and PCR-mix-1-FRT (SC) *Influenza virus A H5N1*). All components of the **eSens Influenza virus A/H5N1 QL PCR kit** are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: Polymerase (TaqF), PCR-mix-1-FRT (SC) *Influenza virus A*, and PCR-mix-1-FRT (SC) *Influenza virus A H5N1* are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-1-FRT (SC) *Influenza virus A* and PCR-mix-1-FRT (SC) *Influenza virus A H5N1* are to be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

Analytical sensitivity of **eSens Influenza virus A/H5N1 QL PCR kit** is no less than 5×10^3 copies/ml.

13.2 Specificity

The analytical specificity of **eSens Influenza virus A/H5N1 QL PCR kit** is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The clinical specificity of **eSens Influenza virus A/H5N1 QL PCR kit** was confirmed in laboratory clinical trials.

14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

15 KEY TO SYMBOLS USED

 REF	Catalogue number		Caution
 LOT	Batch code		Contains sufficient for <n> tests
 IVD	<i>In vitro</i> diagnostic medical device		Use-by Date
 VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C-	Negative control of extraction
 EC REP	Authorized representative in the European Community	C⁺_A , C⁺_{H5} , C⁺_{N1}	Positive controls of amplification
		IC	Internal control

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

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