IVD

For professional use only

eSens NA RespiViral Multiplex Detection Kit

USER MANUAL

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1. INTENDED USE

The **eSens NA RespiViral Multiplex Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **eSens NA RespiViral Multiplex Detection Kit** is designed to detection of the most common causative agents of acute viral respiratory infections by Real-Time PCR method. Samples are human biological materials: nasopharyngeal swabs, oropharyngeal swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm.

Indications for the use:

- The kit is recommended to use in case of presence of ARVI symptoms;
- stay in the centers of infection (for the purpose of early detection of possible infection and prevention of further spread of infection);

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use the **eSens NA RespiViral Multiplex Detection Kit.**

The **eSens NA RespiViral Multiplex Detection Kit** can be used in research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this user manual.

2. METHOD

The implemented method of reverse transcription followed by polymerase chain reaction is based on RNA reverse transcription process and subsequent amplification of cDNA and DNA.

The RNA reverse transcription stage and PCR amplification of cDNA/DNA stage are performed in one test tube, that increases the sensitivity of the method, reduces the likelihood of contamination and reduces the time of the study.

To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The **eSens NA RespiViral Multiplex Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains four target-specific probes bearing reporter fluorescent dyes (Fam, Hex, Rox and Cy5) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The **eSens NA RespiViral Multiplex Detection Kit** includes the Internal control RNA-IC "A", which is intended to assess the quality of the RNA extraction and polymerase chain reaction. DNA probe used for the detection of the specific amplification product includes the fluorescent dyes Fam, Rox or Cy5.

DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. The application of four fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube.

Defined tubes contain additional probe with Rox dye label – "Marker". It tags the strip orientation. Upon completion of run, software defines actual position of the strip (by means of "marker" position) relative to the position preset by the operator. If it mismatches, the software suggests rearrangement of the tubes by default. In accordance with the operator, order can be rearranged and saved in new file. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

№ of tube in a strip	Dye label/detection channel				Color labeling of the buffer
	Fam	Hex	Rox	Cy5	
1	Influenza A virus	RNA-IC	SARS-CoV-2 coronavirus, E, N - genes	Influenza B virus	Blue
2	Human parainfluenza virus type 2	RNA-IC	Human parainfluenza virus type 4	Human coronavirus 229E	Colorless
3	Human bocavirus	RNA-IC	Marker	Human rhinovirus	
4	Human respiratory syncytial virus	RNA-IC	–	Human coronavirus HKU1	
5	Human adenovirus	RNA-IC	–	Human coronavirus NL63	
6	Human coronavirus OC43	RNA-IC	–	Human parainfluenza virus type 3	
7	Human parainfluenza virus type 1	RNA-IC	–	–	
8	Human metapneumovirus	RNA-IC	–	–	

3. CONTENT

The **eSens NA RespiViral Multiplex Detection Kit** contains PCR-mix, RT-PCR-buffer, Enzyme Taq/RT, internal control RNA-IC ”A”, Dilution buffer and positive control sample. The detailed description of content is represented in Table 2.

Table 2. The **eSens NA RespiViral Multiplex Detection Kit** content for ES3310B

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent or blue liquid under waxy white fraction	2880 µL (15 µL per tube)	24 8-tube strips
RT-PCR-buffer	Colorless transparent liquid	3.0 mL (1.0 mL per tube)	3 tubes
Enzyme Taq/RT	Colorless transparent viscous liquid	100 µL	1 tube
Internal control RNA-IC ”A”	Colorless transparent liquid	250 µL	1 tube
Dilution buffer	Colorless transparent liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	320 µL	1 tube
Strip’s caps	24 8-caps		

All components are ready to use and do not require additional preparation for operation.

The **eSens NA RespiViral Multiplex Detection Kit** is intended for single use and designed for 24 tests (defined samples, positive control and negative control)**.**

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Specimen collection swabs: use only dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts;
- Sterile tubes containing transport media
- For bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm: sterile containers with a volume of up to 60 mL.

4.2. NA extraction and PCR

Preamplification-specimen and control preparation area

- Biological safety cabinet class II-III;
- Vortex mixer;
- Refrigerator;
- Nucleic acid extraction kit - follow the manual for the relevant extraction kit. It is possible to use any commercial RNA/DNA isolation kit validated by CE IVD for the specified sample types. Ecoli Dx, s.r.o. recommends to use the ePure Viral NucleicAcid Extraction Kit E2003.
- High speed centrifuge (RCF 12000 - 16000 x g) for 1.5 mL tubes;
- Solid-state thermostat with timer or similar and RNase and DNase free tubes with snap caps, for example Eppendorf Safe-Lock Tubes, or solid-state thermostat maintained a temperature of 65 °C and RNase and DNase free 1.5 mL microcentrifuge tubes with caps;
- Tube rack for 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile) for the preparation of negative control sample (if needed);
- Container for used pipette tips, tubes and other consumables;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- Single channel pipettes (dispensers covering 0.2-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- RNase and DNase free pipette tips for aspirator with trap flask;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area

- UV PCR cabinet;
- Vortex mixer;
- Refrigerator;
- Tube rack for 1.5 mL tubes;
- 1.5 mL microcentrifuge tubes with caps;

- PCR tube rack for 0.2 mL tubes;
- PCR tube rack for strips of eight 0.2 mL tubes;
- Vortex rotor for strips;
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- Powder-free surgical gloves;
- Disinfectant solution;
- Container for used pipette tips, tubes and other consumables.

Post-Amplification – Amplification detection area

- Real-time PCR thermal cycler

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of **eSens NA RespiViral Multiplex Detection Kit**, except the Enzyme Taq/RT, must be stored at temperatures from 2 °C to 8 °C during the storage period. The PCR-mix for amplification must be stored out of light at temperatures from 2 °C to 8 °C during the storage period. The excessive temperature and light can be detrimental to product performance. The Enzyme Taq/RT must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

The kit can be transported in thermal containers with icepacks by all types of covered transport at temperatures corresponding to the storage conditions of the kit components inside the container over the transportation. Transportation is allowed in thermal containers with icepacks by all types of covered transport at temperatures from 2 °C to 25 °C inside the container, but for no longer than 5 days.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit, except the Enzyme Taq/RT, should be stored at temperatures from 2 °C to 8 °C during the storage period; PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period;
- Enzyme Taq/RT should be stored at temperatures from minus 18°C to minus 22 °C during the storage period.

The kit stored in under undue regime should not be used.

An expired the **eSens NA RespiViral Multiplex Detection Kit** should not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **eSens NA RespiViral Multiplex Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

Only specially trained personnel with medical or biological (veterinary) education who have been trained at licensed courses of primary specialization in working with pathogenic microorganisms and who have received additional special training at advanced training courses on molecular and biological methods of diagnostics are allowed to work with the kit of reagents.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain 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 dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The 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. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Use powder-free surgical gloves. Use protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip 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. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. 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 exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Waste materials are disposed of in accordance with local

and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the Master Mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **eSens NA RespiViral Multiplex Detection Kit** is designed to detect NA extracted from the nasopharynx and oropharynx swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm, depending on professional prescription.

Interfering substances

The presence of PCR inhibitors in a sample may cause controversial (uncertain) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

PCR inhibitors are the presence of hemoglobin in the RNA sample as a result of incomplete removal during the extraction of RNA from a biomaterial sample containing an impurity of blood, as well as the presence of isopropyl alcohol and methyl acetate in the RNA sample as a result of incomplete removal of washing solutions during sample preparation.

The maximum concentration of interfering substances, which do not affect the amplification of the laboratory control sample and internal control: hemoglobin – 0.35 mg/mL RNA sample, isopropyl alcohol – 100 µL/mL RNA sample, methyl acetate – 100 µL/mL RNA sample.

Impurities contained in the biomaterial sample, such as mucus, blood, elements of tissue breakdown and inflammation, local medicines, including those that are contained in nasal sprays, etc. should be removed during the NA extraction using sample preparation kits. To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose NA extraction methods that allow to remove PCR inhibitors from the sample as much as possible. It is not recommended to use express methods of NA extraction.

The features of biomaterial sampling:

Each sample of biomaterial should be placed in a separate transport container providing requirements in accordance with the table of guidance.

Sampling procedure is carried out using special sterile disposable instruments – dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts, depending on the source of clinical material in accordance with established procedures.

Nasopharynx swabs

Take the swab with a dry sterile disposable probe into 1.5 mL plastic tubes with transport medium.

Order of taking:

1. Insert the probe carefully along the outer wall of the nose to a depth of 2-3 cm to the lower shell. Then lower the probe down slightly, insert into the lower nasal passage under the lower nasal conch, after a rotational movement remove along the outer wall of the nose.
2. Open the tube.
3. Put the probe into the tube with transport medium and rinse it thoroughly. Avoid spraying of solution.
4. Remove the probe from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the probe. Dispose the used probe.
5. Close the tube tightly and mark it.

Oropharynx swabs

Take the swab with a dry sterile disposable probe into 1.5 mL plastic tubes with transport medium.

Order of taking:

1. Take the swab with a probe with a rotational movement from the surface of the tonsils, palatine arches and the back wall of the pharynx.
2. Open the tube.
3. Put the probe into the tube with transport medium, rotate the probe for 10-15 seconds and rinse it thoroughly. Avoid spraying of solution.
4. Remove the probe from the solution and, by rotating it against the wall of the test tube above the level of the solution, squeeze out the excess liquid. Dispose the used probe.
5. Close the tube tightly and mark it.

Bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate, phlegm

Samples are collected in sterile plastic containers. Transfer 1.0 mL of the sample to a 1.5 plastic test tubes, close the test tube and mark.

For RNA extraction, 100 µL of the sample is used. Do not perform centrifugation as a pretreatment of nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate.

Transportation and storage of the samples in accordance with guidance

Type of the sample	Collecting material requirements	Transportation	Storage conditions before testing	Comments
Nasopharynx and oropharynx swabs	Plastic test tubes and tampons for swabs **	4 °C	≤5 days: 4 °C >5 days *:minus70 °C	Nasopharyngeal and oropharyngeal tampons should be placed in the same tube to increase the viral load
Bronchoalveolar lavage	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *:minus70 °C	A small sample dilution is possible
Endotracheal aspirate, nasopharyngeal aspirate or nasal lavage	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *:minus 70 °C	
Phlegm	Sterile container	4 °C	≤48 hours: 4 °C >48 hours *:minus 70 °C	Make sure that the material is from the lower respiratory tract

* if it is not possible to store samples at minus 70 °C, store samples at minus 20 °C.

** To transport samples, use a transport medium for storing and transporting respiratory swabs or saline solution (if transportation to the laboratory is no more than 24 hours after taking the sample) or a dry swab probe (if transportation to the laboratory is no more than 4 hours after taking the sample).

It is recommended to use transport media containing preservatives, intended for further study of samples by PCR.

Avoid repeated freezing and thawing of samples.

Samples must be transported in accordance with the requirements of the sanitary legislation in relation to pathogenic microorganisms.

8. PROCEDURE

The range of causative agents of ARVI and SARS-CoV-2 viral load can vary widely. In this regard, when performing research in a clinical laboratory, the risk of cross-contamination between samples at all stages of work is a serious danger, especially during aliquoting and RNA extracting. Cross-contamination with high-copy biomaterial can lead to sporadic false-positive results.

To prevent cross-contamination of the biological material in the laboratory, the following rules are recommended:

- it is necessary to conduct a visual assessment of the incoming biomaterial and cull test tubes with broken integrity;
- if possible, it is recommended to analyze samples of patients from a hospital with symptoms of acute infection separately from the rest of the samples (the biological material for screening exposed individuals and patients with mild disease). It is desirable to work with the supposed high-copy samples in a separate box or after working with the supposed low-copy samples;
- it is necessary to use negative control samples, starting from the stage of extracting RNA in each protocol;
- use tips with aerosol filters at all stages of the assay;
- strictly follow the assay procedure, open the Eppendorf test tubes with tweezers or a special opener (do not touch inside the tube cap by the gloved hand); when applying reagents, do not touch inside the test tube by the tip (if this happened, immediately replace the tip).

8.1. NA extraction

It is possible to use any commercial RNA/DNA isolation kit if it is validated by CE IVD for the specified sample types. Ecoli Dx, s.r.o. recommends the use of the ePure Viral NucleicAcid Extraction Kit E2003

8.2. The features of biomaterial preparation

Do not perform centrifugation as a pretreatment of nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage, endotracheal aspirate, nasopharyngeal aspirate.

For RNA extraction, 100 µL of the sample is used.

8.3. The use of control samples at the stage of nucleic acid extraction

Internal control sample

To exclude false negative results of the study and to control the quality of the study, it is necessary to use an internal control sample to the clinical samples at the stage of nucleic acid extraction.

The internal control RNA-IC “A” from the **eSens NA RespiViral Multiplex Detection Kit** should be used as an internal control sample.

The RNA-IC “A” should be used in the amount of 10 µL per sample.

Negative control sample

To exclude false positive results of the study and to control the quality of the study, it is necessary to use a negative control sample from the nucleic acid extraction stage.

Independently of DNA/RNA extraction kit used, a negative control sample should go through all stages of DNA/RNA extraction simultaneously with the RNA extraction from clinical samples.

Physiological saline solution can be used as a negative control sample in volumes as indicated in the instructions for use of extraction kits or negative control sample that is included in the corresponding extraction kit.

8.4. PCR with Reverse Transcription (RT-PCR)

The reagents and tubes should be kept away from direct sun light.

Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.4.1 Mark the required number of strips with paraffin sealed PCR-mix: 1 strip for each sample to be tested, 1 strip for positive control (C+) and 1 strip for negative control (C-).

Example: to test 6 samples, mark 6 strips (one for each sample), one for “C-” and one for “C+”). The resulting number of strips is 8.

8.4.2 Vortex the RT-PCR-buffer and Enzyme Taq/RT thoroughly for 3-5 seconds, then spin for 1-3 seconds.

Enzyme Taq/RT should be got out from the freezer immediately prior to use

8.4.3 Prepare the mixture of RT-PCR-buffer and Enzyme Taq/RT. Add to the one tube:

- 15 x (N+1) µL of RT-PCR-buffer;
- 0.5 x (N+1) µL of Enzyme Taq/RT,
- N is a quantity of tubes in the marked strips.

Example: to test 6 samples, mark 8 strips. Prepare the mixture of RT-PCR-buffer and Enzyme Taq/RT for 65 (64+1) tubes. Mix 975 µL of RT-PCR-buffer and 32.5 µL of Enzyme Taq/RT.

Taking the Enzyme Taq/RT, it is necessary to dip the tip no more than 1.0 mm and observe the rules for dosing viscous liquids. Thoroughly flush the remaining Enzyme Taq/RT from the tip by pipetting at least 5 times.

8.4.4 Vortex the tube with the mixture of RT-PCR-buffer and Enzyme Taq/RT thoroughly for 3-5 seconds, then spin for 1-3 seconds.

Mixture of RT-PCR-buffer and Enzyme Taq/RT must be prepared immediately prior to use and should be used within one hour after preparation. If it is needed, the prepared mixture can be stored at the temperatures from 2 °C to 8 °C but for no longer than one hour.

8.4.5 Add 15 µL of the RT-PCR-buffer and Enzyme Taq/RT mixture into each tube. Avoid paraffin layer break. Close the strips.

8.4.6 Vortex the tubes with samples, “C-“ and “C+” for 3-5 seconds and spin down drops for 1-3 seconds.

Open the tube, add NA sample (or control sample), then close the strip before proceeding to the next RNA sample to prevent contamination. Close the strips tightly. Use filter tips

8.4.7 Add 10 µL of the NA sample into corresponding tubes. Do not add NA into the “C-”, “C+” strips. Avoid paraffin layer break.

8.4.8 Add 10 µL of negative control sample (C-), which passed whole NA extraction procedures into corresponding strip. Add 10 µL of positive control sample (C+) into corresponding strip. Avoid paraffin layer break.

8.4.9 Spin down the strips for 3-5 seconds to collect drops.

8.4.10 Set the strips into the Real-time Thermal Cycler.

8.4.11 Launch the RealTime_PCR application in “Device operation” mode. Upload “AVRI_S_en.ini” file supplied with the kit before first run. Please refer to eQuantia cycler’s user manual for details on working with .ini files. In subsequent runs add corresponding test to the protocol, specify the number and ID’s of the samples, specify the position of the strips in the thermal unit (p. 8.4.10) and run PCR. See Table 4.

Table 4. The PCR program for eQuantia Thermal Cyclers

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	35	20	0	1		Cycle
2	95	5	0	1		Cycle
3	94	0	10	5		Cycle
	64	0	10		v	
4	94	0	5	45		Cycle
	64	0	10		v	
5	80	0	1	1		Cycle
6	10	…	…	Holding		Holding

√ - optical measurement

9. CONTROLS

The **eSens NA RespiViral Multiplex Detection Kit** contains positive control sample. Positive control is a cloned part of the virus genome. It is produced with genetic engineering techniques and characterized by automatic sequencing. The kit includes the Internal control RNA-IC “A”. RNA-IC “A” is intended to assess the quality of the NA extraction and polymerase chain reaction. To reveal possible contamination a negative control is required.

A negative control sample should go through all stages of NA extraction. Physiological saline solution can be used as a negative control sample in volumes indicated in supplied instructions.

For **eSens NA RespiViral Multiplex Detection Kit** the test result is considered valid when:

- the exponential growth of the fluorescence level for the specific product is present, in this case the internal control is not taken into account;
- the exponential growth of the fluorescence level for the specific product is absent and for internal control is present.

For **eSens NA RespiViral Multiplex Detection Kit** the test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control is not observed.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

10. DATA ANALYSIS

Registration of the PCR results is held in automatic mode. Analysis will be performed by Real-Time PCR application. The interpretation should be performed in accordance with Table 5.

Table 5. The interpretation of assay results for control samples

Detection channel				**Interpretation**
Fam	Hex	Rox	Cy5	
Positive control sample				
Cp is specified (Strip test tubes №№1-8)	Cp is not specified	**Cp is specified (Strip test tubes №№1-2)**	**Cp is specified (Strip test tubes №№1-6)**	Positive result The results are valid
Negative control sample				
Cp/Ct is not specified	**Cp is specified**	Cp is not specified	Cp is not specified	Negative result The results are valid

In the samples of human biological material with target viruses NA, the Real-time PCR thermal cycler should register an increase in fluorescence on the corresponding detection channels (Fam, Rox or Cy5), see Table 6. It is necessary to take into account the possibility of the presence in the sample of nucleic acids of several causative agents of acute viral respiratory infections, including those detected in one amplification tube.

In the samples of human biological material free of target viruses NA, the Real-time PCR thermal cycler should register an increase in fluorescence on the Hex (Internal control sample) detection channel, the increase in fluorescence on the Fam, Rox, and Cy5 channels should be absent.

The results are considered as unreliable (Invalid) if there is no exponential increase in fluorescence on the Fam, Rox, and Cy5 channels (specific product) and on the Hex channel (Internal control sample).

Table 6. The interpretation of assay results for PCR

Detection channel				Interpretation
Fam	Hex	Rox	Cy5	
Strip test tube №1				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Influenza A virus RNA is detected**
Cp is not specified	Is not considered	**Cp is specified**	Cp is not specified	**SARS-CoV-2 RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Influenza B virus RNA is detected**
Strip test tube №2				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human parainfluenza virus type 2 RNA is detected**
Cp is not specified	Is not considered	**Cp is specified**	Cp is not specified	**Human parainfluenza virus type 4 RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human coronavirus 229E RNA is detected**
Strip test tube №3				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human bocavirus DNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human rhinovirus RNA is detected**
Strip test tube №4				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human respiratory syncytial virus RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human coronavirus HKU1 RNA is detected**
Strip test tube №5				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human adenovirus DNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human coronavirus NL63 RNA is detected**
Strip test tube №6				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human coronavirus OC43 RNA is detected**
Cp is not specified	Is not considered	Cp is not specified	**Cp is specified**	**Human parainfluenza virus type 3 RNA is detected**
Strip test tube №7				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human parainfluenza virus type 1 RNA is detected**
Strip test tube №8				
Cp is specified	Is not considered	Cp is not specified	Cp is not specified	**Human metapneumovirus RNA is detected**
For all test tubes				
Cp is not specified	**Cp is specified**	Cp is not specified	Cp is not specified	Target viruses RNA is not detected
Cp is not specified	Cp is not specified	Cp is not specified	Cp is not specified	Unreliable result

Unreliable results may be caused by the presence of inhibitors in the nucleic acid preparation obtained from the clinical material, errors in the pre-analytical stage, incorrect implementation of the analysis Protocol, non-compliance with the temperature mode of amplification, etc. In this case, either re-staging of reverse transcription and polymerase chain reaction, or re-extracting of the nucleic acid

preparation, or re-collect of clinical material (performed sequentially) is required.

When the expressed growing fluorescence (Cp is not specified) through the Fam, Rox, or Cy5 channels is not expressed for positive control (C+), the results of whole series are considered false. It is required to repeat the whole test.

When the expressed growing fluorescence (Cp is specified) through the Fam, Rox, or Cy5 channels is expressed for negative control (C-), the results of whole series are considered false. It is required to eliminate contamination.

A single negative test result, especially if it is a sample from the upper respiratory tract, does not exclude infection.

Negative results should not be used as the sole basis for making a decision about the treatment of patients.

If in the samples of human biological material the Real-time PCR thermal cycler registers an increase in fluorescence for the specific product earlier than 25 cycle for Cp, this indicates a high initial NA concentration of the corresponding pathogen. In this case, it is possible to obtain a false negative result during mixed infection for a pathogen whose NA is present in a low concentration. To exclude false negative results, it is recommended to repeat RT-PCR for the extracted NA preparation using the kit for individual detection of the corresponding virus.

11. SPECIFICATIONS

a. The analytical specificity of the **eSens NA RespiViral Multiplex Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

Since it is impossible to exclude the occurrence of new mutations in the genome of the SARS-CoV-2 coronavirus, two genome sites were selected as targets to improve the reliability of diagnostics: the N and E genes sites.

In the samples of human biological material with target viruses NA, the detecting amplifier should register an increase in fluorescence on the corresponding detection channels.

In the samples of human biological material free of target viruses NA, the detecting amplifier should register an increase in fluorescence on the Hex/Yellow detection channel, the increase in fluorescence on the Fam, Rox, and Cy5 channels should be absent.

There are not cross-nonspecific reactions of each of the oligonucleotide systems included in the kit in relation to viruses determined by other systems.

There are not non-specific positive results of amplification DNA of *Mycoplasma pneumoniae, Streptococcus pneumoniae, Chlamydophila pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, Moraxella catarrhalis, Bordetella pertussis, Bordetella parapertussis*, as well as human DNA in concentrations up to 1.0×10^8 copies/mL of the sample.

b. Analytical sensitivity is 10 copies of NA per amplification tube for Influenza A virus, Influenza B virus, SARS-CoV-2 coronavirus and 20 copies of NA per amplification tube for other viruses. Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS).

12. TROUBLESHOOTING

Table 7. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error RT-PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	RT-PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

Telefon: tel.: +420 325 209 912

E-mail: ecolidx@ecolidx.com

13. QUALITY CONTROL

Ecoli Dx, s.r.o. declares that the above mentioned products meet the provision of the Council Directive 98/79/EC for In vitro Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service with quality issues of **eSens NA RespiViral Multiplex Detection Kit**:

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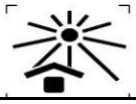
14. KEY TO SYMBOLS

	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limitation		Consult instructions for use
	Sufficient for		Catalogue number
	Use by		Manufacturer
	Batch code		Keep away from sunlight
	Caution		Version
	Negative control		Positive control
	Non-sterile		Do not reuse