For professional use only

# eSens NA Mycosis Multiplex Detection Kit

# USER MANUAL

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REF ES3811B

VER 1.1

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## 1. INTENDED USE

The **eSens NA Mycosis Multiplex Detection Kit** is intended for research and diagnostic applications. The **eSens NA Mycosis Multiplex Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **eSens NA Mycosis Multiplex Detection Kit** is designed for detection and typing of pathogens causing mycoses from genus Candida, Malassezia, Saccharomyces and Debaryomyces in DNA material obtained from human biological samples, catheter and endotracheal tube washings, and fungal cultures with an aid of Polymerase Chain Reaction (PCR) method.

Samples are human biological material (blood, phlegm, urine, smears/scrapes from respiratory tract, gastrointestinal and urogenital tracts, faeces, bioptates), catheter and endotracheal tube washings, and fungal cultures.

Indications for the use:

- a suspicion for candidiasis, candidemia, candiduria and Candida carrier state;
- monitoring of the dynamic of colonization normally non-sterile loci, lesions and catheters with fungi;
- infectious control in patients including risk groups;
- identification of fungal species in fungal cultures.

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

The **eSens NA Mycosis Multiplex Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and 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

Method: polymerase chain reaction (PCR) with detecting of the results in real time; semi-quantitative multiplex analysis.

The implemented PCR method is based on amplification of a target DNA sequence. To increase the sensitivity and specificity of amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by paraffin layer. 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 Mycosis Multiplex Detection Kit** is based on fluorescent modification of the PCR method.

The PCR-mix contains target-specific probes, each of them bearing reporter fluorescent dyes and quencher molecules. When specific product is formed, DNA probes are disintegrated and the quencher molecule stops affecting the fluorescent dye. Thus the level of fluorescence increases and it is detected by the thermocycler data collection unit. As a result of probe activation fluorescence increases proportionally to target sequence amplification. The amount of disintegrated probes increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction in real time with a Real-time PCR thermo cycler.

DNA probes for target sequences of fungal pathogens contain fluorescent dyes Fam and Cy5. DNA probes for the detection of amplification products of internal control (IC) and sample intake control (SIC) contain 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.

The PCR-mix in tube №3 contains 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 and in case of mismatch warns an operator. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

| № of a tube in a strip | Detection channel | | | | | Color of the PCR-mix |
|---|---|---|---|---|---|---|
| | Fam | Hex | Rox | Cy5 | Cy5.5 | |
| 1 | Meyerozyma guilliermondii (C.guilliermondi) | IC | - | - | - | Blue |
| 2 | Candida albicans | IC | - | Pichia kudriavzevii (C.krusei) | - | Colorless |
| 3 | Saccharomyces cerevisiae | IC | Marker | Candida auris | - | |
| 4 | Candida tropicalis | IC | - | Clavispora lusitaniae (Candida lusitaniae) | - | |
| 5 | Debaryomyces hansenii (C.famata) | IC | - | Candida dubliniensis | - | |
| 6 | Candida glabrata | IC | - | Candida parapsilosis | - | |
| 7 | Malassezia spp. | IC | - | Malassezia furfur | - | |
| 8 | Kluyveromyces marxianus (C.kefyr) | SIC | - | - | - | |

## 3. CONTENT

The **eSens NA Mycosis Multiplex Detection Kit** contain paraffin sealed PCR-mixes, Taq-polymerase solution, mineral oil and positive control. The detailed description of content is represented in Table 2.

Table 2. The **eSens NA Mycosis Multiplex Detection Kit** content for ES3811B

| Reagent | Description | Total volume | Amount |
|---|---|---|---|
| Paraffin sealed PCR-mixes | Colorless or blue transparent liquid under waxy white fraction | 3840 µL (20 µL per tube) | 24 8-tube strips |
| Taq-polymerase solution | Colorless transparent liquid | 2.0 mL (500 µL per tube) | 4 tubes |
| Mineral oil | Colorless transparent viscous oily liquid | 4.0 mL (1.0 mL per tube) | 4 tubes |
| 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 Mycosis Multiplex Detection Kit** is intended for single use and designed for 24 tests (no more than 20 defined samples, one positive control and one negative control).

## 4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

### 4.1. Specimen collection

- Sterile single use swabs, single-use sterile flasks and sterile containers to collect clinical material;
- Sterile tubes containing transport medium or physiological saline solution for the transportation of the sample;
- For blood collection: 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

### 4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;
- Nucleic acid extraction kit - follow the manual for the relevant extraction kit.

  It is possible to use any commercial RNA/DNA extraction kit validated by CE IVD for the specified sample types. Ecoli Dx, Ltd. recommends use of the ePure Bacterial DNA Extraction Kit E2006
- High speed centrifuge (RCF 16000 x g);
- Solid-state thermostat (temperature range 40-95 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Single channel pipettes (dispensers covering 20-1000 µL volume range);

- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL)
- RNase and DNase free pipette tips for aspirator with trap flask;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Vortex mixer;
- Vortex rotor for 0.2 ml strips;
- Refrigerator;
- Tube rack for 0.2 mL strips and 1.5 mL tubes;
- 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);
- Container for used pipette tips, tubes and other consumables.
- Powder-free surgical gloves;
- Disinfectant solution;

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 Mycosis Multiplex Detection Kit** must be stored at temperatures from 2°C to 8°C during the storage period. The PCR-mixes for amplification must be stored out of light during the storage period. The excessive temperature and light can be detrimental to product performance.

The kit transportation can be held in thermal containers with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components. It is allowed to transport the kit in thermal containers with ice packs by all types of roofed transport at temperatures inside the container from 2 °C to 25 °C for no more than 5 days.

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

- components of the kit should be stored at temperatures from 2°C to 8 °C during the storage period;
- PCR-mixes for amplification should be stored at temperatures from 2°C to 8 °C and out of light during the storage period;

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

An expired **eSens NA Mycosis 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 Mycosis 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 personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

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, 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. Work surfaces, as well as rooms where PCR is performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work. 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 PCR-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 Mycosis Multiplex Detection Kit** is designed for the detection and typing of fungal infectious agents from genera Candida, Malassezia, Saccharomyces and Debaryomyces: Meyerozyma guilliermondii (C. guilliermondii), Candida albicans, Pichia kudriavzevii (C.krusei), Saccharomyces cerevisiae, Candida auris, Candida tropicalis, Clavispora lusitaniae (C.lusitaniae), Debaryomyces hansenii (C.famata), Candida dubliniensis, Candida glabrata, Candida parapsilosis, Malassezia spp., Kluyveromyces marxianus (C.kefyr), Malassezia furfur in DNA material obtained from human biological samples (blood, phlegm, urine, smears/scrapes from respiratory tract, gastrointestinal and urogenital tracts, faeces, bioptates), catheter and endotracheal tube washings, and fungal cultures, 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 DNA sample as a result of incomplete removal during the extraction of DNA from a biomaterial sample containing an impurity of blood, as well as the presence of isopropyl alcohol and methyl acetate in the DNA 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 DNA sample, isopropyl alcohol – 100 µL/mL DNA sample, methyl acetate – 100 µL/mL DNA sample. Concentration of interfering substances in the blood samples at which PCR inhibition is not observed: bilirubin– 684 µmoL/L, cholesterol – 13 µmoL/L, triglycerides – 37 µmoL/L.

Impurities contained in the biomaterial sample, such as mucus, blood, elements of tissue breakdown and inflammation, local medicines, including those that are contained in vaginal suppositories, talc, spermicide, etc. should be removed during the DNA 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 DNA extraction methods that allow to remove PCR inhibitors from the sample as much as possible. It is not recommended to use express methods of DNA extraction.

**General requirements**

PCR analysis refers to direct methods of laboratory research; therefore the collection of biological material must be carried out from the site of infection localization. Professional prescription is required to localize the place of sampling. The decision must be based on patient's complaints and clinical signs, and made by the physician in charge. To interpret results successfully and robustly, a high quality of sample and appropriate conditions of storage, transport, and handling are required.

Inappropriate sampling may result in doubtful results and sample collection may need to be

repeated.

**Sample collection**

Before DNA extraction pre-processing of biological material samples is needed.

**Blood**

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant. After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.

It is not allowed to use heparin as an anticoagulant.

**Phlegm**

Sample taking is made in amount no less than 1.0 mL into single-use graduated sterile flasks with wide neck and screwing caps with volume no less than 50 mL.

After sample collection, flask is tightly screwed and marked.

**Urine**

The first portion of morning urine in the amount of 20–30 mL is selected for the analysis. The urine is taken into a special dry sterile container with volume of up to 60 mL, equipped with a hermetical screw-cap.

After the urine collection, container is tightly screwed and marked.

**Smears/scrapes from respiratory tract, gastrointestinal and urogenital tracts**

Sample taking is made with special sterile single-use tools – probes, cytobrushes, swabs depending on the source of biological material according to established procedure.

In case of pregnancy the use of cytobrushes for genitourinary smears sampling is contraindicated.

Order of taking:

1. Open the tube with a transport medium.

2. Scrape epithelial cells from the corresponding biotope (i.e. respiratory tract, gastrointestinal and urogenital tracts) with a sterile swab.

3. Put the swab into the tube with transport medium and rinse it thoroughly. Avoid spraying of solution.

4. Remove swab from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the swab.

5. Close the tube tightly and mark it.

**Faeces**

Samples of faeces with mass (volume) 1.0-3.0 g (1.0-3.0 mL) are transferred to a sterile dry flask by a single-use filtered pipette tip or single-use shovel.

After sample collection the flask is tightly closed with a lid and marked.

**Bioptates sampling**

Bioptates are transferred to a 1.5 mL tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR.

After sample collection the tube is tightly closed and marked.

**Washings from parts of intravenous catheter**

Cut with sterile scissors 5.0-10 mm of a catheter tip and place it into a 1.5 mL tube. Close the tube and mark it.

**Smears from endotracheal tubes**

Sample taking is made from a surface of endotracheal tube with special sterile single-use probes.

After sample collection, place the probe into 1.5 mL tube with a transport medium for transportation and storage of biological material for PCR and then rotate for 10-15 seconds, avoiding splashing of the liquid. Then 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, close the test tube and mark it.

**Washings from endotracheal tubes**

Sample taking is made in single-use 50 mL tubes. After sample collection, close the tube tightly and mark it. Invert the tube 3-5 times to mix the material.

**Bacterial cultures sampling**

Sample taking from liquid and solid media is made with single-use microbiological loop or spreader. Place a sole colony of cells or 100 µL of liquid media in single-use 1.5-2.0 mL tube with 500 µL of sterile saline solution.

Close the tube tightly and mark it.

**Transportation and storage of the samples**

**Blood**

Blood samples are allowed to transport and store:

- at room temperature from 20 °C to 25 °C no more than 2 hours;
- at temperature from 2 °C to 8 °C no more than 6 hours from taking the material.

The whole blood cannot be frozen.

**Phlegm**

Phlegm samples can be transported and stored:

- at room temperature from 18 °C to 25 °C no more than 6 hours;
- at temperature from 2 °C to 8 °C no more than 3 days.

**Native or preprocessed urine samples**

Native or preprocessed urine samples can be transported and stored:

- at temperature from 2 °C to 8 °C no more than 1 day
- at temperature from minus 18 °C to minus 20 °C no more than one week

- at minus 70 °C – 6 months

Only one freezing-unfreezing of the material is allowed.

**Smears/scrapes from respiratory tract, gastro-intestinal and urogenital tracts**

Smears/scrapes from respiratory tract, gastrointestinal and urogenital tract must be transported and stored according to the instructions for DNA extraction kits.

**Native faeces samples**

Native faeces samples can be transported and stored:

- at room temperature from 18 °C to 25 °C no more than 6 hours;
- at temperature from 2 °C to 8 °C no more than 3 days.

**Bioptates sampling, parts of intravenous catheters, smears and washings from endotracheal tubes, bacterial cultures**

Bioptates sampling, parts of intravenous catheters, smears and washings from endotracheal tubes, bacterial cultures can be transported and stored:

- at temperature from 2 °C to 8 °C no more than 1 day;
- at temperature from minus 18 °C to minus 20 °C no more than one week;
- at minus 70 °C – 6 months.

 Only one freezing-unfreezing of the material is allowed.

**Sample preparation**

It is necessary to perform pretreatment before DNA extraction. It is allowed to use any kits of reagents registered as a medical device and recommended by manufacturers for the extraction of DNA from the corresponding types of biomaterial.

**Blood**

Preparation of blood samples are made according to the instructions for DNA extraction kits.

**Phlegm**

Method 1

1. Put approximately 500 µL of biological sample into sterile 1.5 mL tube and close it tightly.
2. Add to the sample an equal volume of 10% triple-substituted sodium phosphate $x12H_2O$, close it tightly and mix intensively.
3. Incubate the mixture at 37 °C for 18–24 hours, then neutralize with 1M HCl (down to pH 6.8–7.4).
4. Centrifuge 1.5 mL tube at 100-200 x g for 20 minutes.
5. Take out the supernatant into the 5% solution of chloramine for disinfection.
6. Add 500 µL of distilled water to precipitate, mix by pipetting and put to the new 1.5 mL tube.
7. Centrifuge the tube at 16000 x g for 10 minutes.

Remove the supernatant, leaving the volume of precipitate+liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

Method 2

1. Add mucolysin to the container with sample in the 5:1 ratio (5 parts of mucolysin to 1 part of phlegm), referring to container calibrations.

2. Close the container, mix the container content and incubate it at room temperature for 20–30 minutes, shake the container every 2-3 minutes.

**Urine**

1. Transfer 1.0 mL of the sample to the 1.5 mL tube.
2. Centrifuge the tube at 16000 x g for 10 minutes.
3. Remove the supernatant completely.
4. Add 1.0 mL of sterile saline solution to the precipitate.
5. Centrifuge the tube at 16000 x g for 10 minutes.
6. Remove the supernatant, leaving the volume of precipitate+liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

**Smears/scrapes from respiratory tract, gastrointestinal and urogenital tracts, smears from endotracheal tubes, bacterial cultures from liquid and solid media**

1. Centrifuge the tube at 16000 x g for 10 minutes.
2. Remove the supernatant, leaving the volume of precipitate+liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

1. Put approximately 0.1-0.2 g (mL) of faeces into the 1.5 mL tube with 1.0 mL of sterile saline solution.
2. Vortex the tube for 5-10 seconds.
3. Further processing of the suspension is carried out in accordance with the instruction for the DNA extraction kit from the corresponding biomaterial.

**Bioptates sampling**

1. Centrifuge the tube at 16000 x g for 10 minutes.
2. Remove the supernatant, leaving the volume of precipitate+liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

**Washings from endotracheal tubes**

1. Transfer 1.0 mL of biomaterial into 1.5 mL tube using an automatic dispenser with filtered pipette tip.
2. Centrifuge the tube at 16000 x g for 10 minutes.
3. Remove the supernatant, leaving the volume of precipitate+liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

**Fragments of intravenous catheters (only with PREP-NA DNA/RNA Extraction Kit)**

1. Add 100 µL of distilled water or sterile saline solution in the tube with a fragment of catheter.
2. Vortex the tube for 3-5 seconds and spin down the drops for 1-3 seconds on vortex mixer.
3. Add in the tube 300 µL of lysis buffer from **PREP-NA DNA/RNA Extraction Kit**.
4. Vortex the tube for 3-5 seconds and spin down the drops for 1-3 seconds on vortex mixer.
5. Termostate the tube on 65 °C for 15 minutes.
6. Spin down the drops for 1-3 seconds on vortex mixer and transfer the supernatant in a new 1.5 mL tube.

Further DNA extraction is carried out starting with the step of adding a precipitation solution.

Further processing of the samples should be done according to instructions for DNA extraction kits.

## 8. PROCEDURE

### DNA extraction from biological material

DNA extraction is carried out in accordance with the instruction for extraction kits. DNA extraction kits for subsequent usage of DNA in PCR are recommended. The quality control of the extraction is carried out by the system of internal control (IC).

For fragments of intravenous catheters **ePure Bacterial DNA Extraction Kit (E2006)** is recommended.

Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control sample in volumes as indicated.

### Assay procedure

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!

8.1 Mark one strip with paraffin sealed PCR-mix for each test sample, one for positive control (C+) and one for negative control (C-).

**Example:** to test 2 samples, mark 4 strips - 2 strips for the samples, 1 strip for “C-” and 1 strip for “C+”. See Table 3 for reference.

Table 3. Example of tube marking for PCR procedure

| **Samples** | **Number of strip** | **Number of tube in the strip** |
|---|---|---|
| Sample 1 | strip 1 | tubes 1-8 |
| Sample 2 | strip 2 | tubes 1-8 |
| C- | strip 3 | tubes 1-8 |
| C+ | strip 4 | tubes 1-8 |

8.2 Vortex the tube with Taq-polymerase solution for 3-5 seconds, then spin briefly for 1-3 seconds to collect the drops.

8.3 Add 10 µL of Taq-polymerase solution into each tube of the strip. Avoid paraffin layer break.

8.4 Add one drop (~20 µL) of mineral oil into each strip tube. Close the tubes.

8.5 Vortex the tubes with DNA samples, positive control sample and negative control sample for 3-5 seconds, then spin down drops for 1-3 seconds.

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

8.6 Add 5.0 µL of DNA sample into corresponding strip tubes. Do not add DNA into the “C-“, “C+” strip tubes. Avoid paraffin layer break.

8.7 Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure and positive control (C+) into corresponding tube. Avoid paraffin layer break.

8.8 Spin the strips briefly for 1-3 seconds on vortex mixer.

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

8.10 Launch the RealTime_PCR application in “Device operation” mode. Upload the «Mycosis_screen_en.ini» file supplied with the kit before first run. Please refer to eQuantia thermal 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. 8.9) 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 | 80 | 0 | 30 | 1 | | Cycle |
| | 94 | 1 | 30 | | | |
| | | | | | | |
| 2 | 94 | 0 | 30 | 5 | | Cycle |
| | 64 | 0 | 15 | | √ | |
| | | | | | | |
| 3 | 94 | 0 | 10 | 45 | | Cycle |
| | 64 | 0 | 15 | | √ | |
| | | | | | | |
| 4 | 94 | 0 | 5 | 1 | | Cycle |
| | | | | | | |
| 5 | 10 | | … | Holding | | Holding |
| √ - optical measurement | | | | | | |

## 9. CONTROLS

The **eSens NA Mycosis Multiplex Detection Kit** contains positive control sample. Positive control is a cloned part of the fungal genome. It is produced with genetic engineering techniques and characterized by automatic sequencing. The PCR-mix from the kit includes the Internal control. IC is an artificial plasmid intended to assess the quality of PCR performance. To reveal possible contamination a negative control is required.

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

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 absence and for internal control is present.

The test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control are 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 are held in automatic mode. The graph will show the fluorescence dependence of the number of cycle on all detecting channels for each tube in the thermoblock. The table will show the sample ID, threshold cycles (Cp) and decimal logarithms of concentrations (Lg) of target DNA copies in 1 mL of the DNA preparation through the corresponding channels and interpretation of the amplification results («+» or « - »). It is possible to create and print a report based on the analysis results.

After the end of the amplification program the software compares predetermined order of tubes with the real localization of the Rox marker and in case of mismatch warns an operator. In this case the operator should check the localization of the strips in a thermoblock (the first tube is marked by a blue buffer) and correct identifiers of tubes in the protocol.

Analyzing the results, the values of sample intake control (SIC, tube №8 of the strip, Hex channel) and internal control (IC, tubes №1-7, Hex channel) must be taken into account:

1. To control the sample intake containing human cells, the parameter SIC (the sufficient amount of human DNA) is used. The SIC value less than 3.0 in case of absence of specific results in all tubes of the strip is considered as insufficient amount of biomaterial. In this case resampling is recommended.

In case of analyzing biomaterial that does not contain human DNA (fragments of intravenous catheters, smears and washings from endotracheal tubes, bacterial cultures), the SIC value is not taking into account.

2. To access the quality of DNA extraction, Internal control is used. If the IC is not present in one or more tubes in the strip and at the same time there are the absence of specific positive results in these tubes, the result in these tubes is considered invalid due to incorrect conduction of PCR. In this case repeating of PCR amplification, or reextraction of DNA, or resampling is needed (performed sequentially).

3. In the samples containing DNA of detected pathogens the software detects positive result on the corresponding detection channel (Fam or Cy5) in a corresponding tube. In the result table in the line with the name of this pathogen the result of the qualitative analysis (“+”), the value of threshold cycle (Cp) and the decimal logarithm of concentration (Lg, the Lg of the number of copies of DNA target in 1 mL of sample) will be indicated. The interpretation of the result is “detected (N Lg)”.

4. In the samples not containing DNA of detected pathogens the software detects negative result on the corresponding detection channel (Fam or Cy5) in a corresponding tube. In the result table in the line with the name of this pathogen the result of the qualitative analysis (“-”) will be indicated. The interpretation of the result is “not detected”.

5. In the tube №5 of the strip the value of Lg≤2.5 on Fam detection channel is not taking into account by the software. In the result table in the "Result" column the result of qualitative analysis (“-“) will be indicated. The interpretation of the result is “not detected”.

6. In the tube №7 of the strip the value of Lg≤2.5 on Fam detection channel is not taking into account by the software in case of the absence the exponential increase of fluorescence on Cy5 detection channel. In the result table in the "Result" column the result of qualitative analysis (“-“) will be indicated. The interpretation of the result is “not detected”.

7. In the tube №8 of the strip the value of Lg≤2.0 on Hex detection channel is not taking into account by the software. In the result table in the "Result" column the result of qualitative analysis (“-“) will be indicated. The interpretation of the result is “not detected”.

8. For positive and negative control samples the results must correspond to those from the Table 5. In the negative control sample the IC value must be no less than 3.5. In the positive control sample the IC value is not taking into account.

9. If results for negative control sample differ from those in table 4, the results of the whole series are considered invalid. In this case decontamination is required.

10. The positive control sample is needed to access the efficiency of PCR reaction. In case of adherence to all conditions of reaction, the amount of target DNA in the positive control sample must correspond to the Table 5. If results for positive control sample differ from those in table 4, repeat of amplification of the whole series is required.

Table 5. The results of the test for positive and negative control samples

| **№ of the tube in a strip** | **Pathogen** | **C- (Lg)** | **C- result** | **C+ (Lg)** | **C+ result** |
|---|---|---|---|---|---|
| 1 | Meyerozyma guilliermondii | - | - | 3.5-5.5 | + |
| 2 | Candida albicans | - | - | 3.5-5.5 | + |
| | Pichia kudriavzevii | - | - | 3.5-5.5 | + |
| 3 | Saccharomyces cerevisiae | - | - | 3.5-5.5 | + |
| | Candida auris | - | - | 3.5-5.5 | + |
| 4 | Candida tropicalis | - | - | 3.5-5.5 | + |
| | Clavispora lusitaniae | - | - | 3.5-5.5 | + |
| 5 | Debaryomyces hansenii | - | - | 3.5-5.5 | + |
| | Candida dubliniensis | - | - | 3.5-5.5 | + |
| 6 | Candida glabrata | - | - | 3.5-5.5 | + |
| | Candida parapsilosis | - | - | 3.5-5.5 | + |
| 7 | Malassezia spp. | - | - | 3.5-5.5 | + |
| | Malassezia furfur | - | - | 3.5-5.5 | + |
| 8 | Kluyveromyces marxianus | - | - | 3.5-5.5 | + |
| | SIC | - | - | 3.5-5.5 | + |

According to analysis of the results a report can be made up and printed.

## 11. SPECIFICATIONS

a. The analytical **specificity** of the **eSens NA Mycosis 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.

The samples of human biological material with DNA of the detected mycosis pathogens are to be registered positive for specific product through the declared detection channels.

The samples of human biological material free of DNA of the detected mycosis pathogens are to be registered negative for specific product through the declared detection channels.

In the samples of biological material, containing human genomic DNA, the detecting amplifier should register a positive result of SIC amplification.

In the samples of biological material, not containing human genomic DNA, the detecting amplifier should register a negative result of SIC amplification.

For each test in the kit, there are not cross non-specific results with all other tests from the kit and non-specific positive results of amplification in the presence of other microorganisms or human DNA in concentration up to $1.0\times10^8$ copies/mL of the sample.

b. Analytical **sensitivity** of the **eSens NA Mycosis Multiplex Detection Kit** is 5 copies of DNA per amplification tube ($1.0x10^3$ copies/mL DNA sample). Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS).

Sensitivity depends on the type of biomaterial, the extraction kit used for DNA extraction and the final elution volume (dilution) of the extracted DNA.

c. Diagnostic characteristics

Number of samples (n) - 429;
Diagnostic sensitivity (95% CI) - 100% (98.6-100%);
Diagnostic specificity (95% CI) – 100% (97.9-100%).

## 12. TROUBLESHOOTING

Table 6. Troubleshooting

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

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

Tel: 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 official representative in EU by quality issues of **eSens NA Mycosis Multiplex Detection Kit**:

Technical support E-mail: ecolidx@ecolidx.com,

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## 14. KEY TO SYMBOLS

| | | | |
|---|---|---|---|
| IVD | *In vitro* diagnostic medical device | | Date of manufacture |
| | Temperature limitation | | Consult instructions for use |
| Σ | Sufficient for | REF | Catalogue number |
| | Use by | | Manufacturer |
| LOT | Batch code | | Keep away from sunlight |
| | Caution | VER | Version |
| CONTROL − | Negative control | CONTROL + | Positive control |
| EC REP | Authorized representative in the European Community | NON STERILE | Non-sterile |