DNA-TECHNOLOGY

710 2021-08-10

CE IVD

For professional use only

MycosoScreen REAL-TIME PCR Detection Kit

USER MANUAL

EC REP

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels,Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

"DNA-Technology Research & Production", LLC

Russia, 142281, Moscow Region,

Protvino, 20 Zheleznodorozhnaya Street,

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com
http://www.dna-technology.ru

Customer service department
E-mail: hotline@dna-technology.ru

REF R1-P023-S3/5EU

VER 710.2021.08.10

TABLE OF CONTENTS

1. INTENDED USE

The ***MycosoScreen*** **REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The ***MycosoScreen*** **REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The ***MycosoScreen*** **REAL-TIME PCR 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.

The ***MycosoScreen*** **REAL-TIME PCR Detection Kit** is intended for detection and typing of fungal infectious agents from genus 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. Samples are human biological material (blood, phlegm, urine, swabs/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 ***MycosoScreen*** **REAL-TIME PCR Detection Kit.**

The ***MycosoScreen*** **REAL-TIME PCR 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 in working with pathogenic microorganisms 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 result of the test is represented as decimal logarithm of the number of DNA copies in 1 ml of DNA sample.

The implemented PCR method is based on amplification of a target DNA sequence. The process of amplification includes repeating cycles of thermal DNA denaturation, annealing of primers with complementary sequences and their extension by Taq-polymerase.

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 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 ***MycosoScreen*** **REAL-TIME PCR 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 reagents in tubes 1-7 contain internal control (IC) for the estimation of the PCR efficiency.

The amplification mix in the tube №8 contains the reagents for the detection of SIC that is required for the analysis of the efficiency of DNA extraction from human biological material and allows to determine if the amount of DNA is sufficient for the analysis.

The tube №3 contains the oligonucleotide with fluorescent dye Rox – a marker. It is used by detecting thermocycler for the detection of strip's position in the thermoblock. After the end of the amplification program the software compares predetermined order of tubes with the real localization of the marker ROX and in case of mismatch warns an operator.

Table 1 shows the detection channels and color marking of the mixes.

Table 1. Detection channels and color marking of the mixes

№ of a tube in a strip	Detection channel					Color of the mix
	Fam	Hex	Rox	Cy 5	Cy 5.5	
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	-	-	-	

The automatic analysis available on "DNA-Technology" made instruments: **DT*lite*** or **DT*prime* REAL-TIME Thermal Cyclers** for ***MycosoScreen* REAL-TIME PCR Detection Kit** (see the catalogue at www.dna-technology.com/en to see available supply options).

The current version of the software is available for download at http://dna-technology.com/software.

3. CONTENT

The ***MycosoScreen*** **REAL-TIME PCR Detection Kit** content is represented in Table 2.

Table 2. The MycosoScreen REAL-TIME PCR Detection Kit content, package S (standard) for R1-P023-S3/5EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	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	500 µL	4 tubes
Mineral oil	Colorless transparent viscous oily liquid	1,0 µL	4 tubes
Positive control	Colorless transparent liquid	320 µL	1 tube
Strip's caps	24		

All components of the kit are ready to use and do not require any preparation to work.

The ***MycosoScreen*** **REAL-TIME PCR 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

- Specimen collection: use sterile single use probes, single-use sterile flasks and sterile containers to collect clinical material;
- Use specimen transport medium or physiological saline solution for the transportation of the sample;
- Blood specimen collection: use Vacuette type tubes, containing ethylenediaminetetraacetic acid disodium salt (EDTA) or sodium citrate anticoagulant.

4.2. RNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;
- Nucleic acid extraction kit (The DNA extraction from washings from parts of intravenous catheters is recommended to be carried out using "DNA-Technology" made PREP-NA DNA/RNA Extraction Kit REF P-002/1EU);
- High speed centrifuge (RCF 16000 x g);
- "DNA-Technology" made solid-state programmable thermostat TT-1 or similar with clump lid and RNase and DNase free 1.5 mL microcentrifuge tubes with caps, or solid-state thermostat maintained temperature from 40 °C to 95 °C and RNase and DNase free 1,5 mL tubes with snap caps, for example Eppendorf Safe-Lock Tubes;
- PCR tube rack for 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Container for used pipette tips;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;

- Single channel pipettes (dispensers covering 1.0 -1000 µl);
- RNase and DNase free filtered pipette tips for semi-automatic pipettes (volume 20 µL, 200 µL, 1000 µL)
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area

- UV PCR cabinet;
- Vortex mixer;
- Vortex rotor for 0.2 ml strips (if needed);
- Refrigerator;
- PCR tube rack for 0.2 mL tubes or strips;
- Single channel pipettes (volume 2-20 µl, 20-200 µl, 200-1000 µl);
- 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.

Post-Amplification – Amplification detection area

- Real-time PCR thermal cycler ("DNA-Technology" made "DTprime"[1] , "DTlite"[2] or "DT-96")

Software:

The most recent version of the DT thermal cyclers software can be downloaded from http://dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

All components of ***MycosoScreen*** **REAL-TIME PCR Detection Kit** 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 during the storage period. The excessive temperature and light can be detrimental to product performance.

The kit can be transported in thermal containers with icepacks by all types of roofed transport at temperatures from 2°C to 8 °C over the transportation. Transportation is allowed in thermal containers with icepacks by all types of covered transport at a temperatures up 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 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;

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

An expired ***MycosoScreen*** **REAL-TIME PCR Detection Kit** should not be used.

[1] Only models 4M1; 4M3; 4M6; 5M1; 5M3; 5M6; 6M1; 6M3; 6M6
[2] Only models 4S1; 4S2; 5S1; 5S2; 6S1; 6S2

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

The conformity of the ***MycosoScreen*** **REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the ***MycosoScreen*** **REAL-TIME PCR Detection Kit**.

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

Phone: +7(495)640.16.93,

Phone/Fax: +7(495)640.17.71.

E-mail: http://dna-technology.com/support.

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 coming in contact with the biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121ºC 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 dispensers, 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.

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 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 breach;
- 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 ***MycosoScreen*** **REAL-TIME PCR 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, swabs/scrapes from respiratory tract, gastrointestinal and urogenital tracts, faeces, bioptates), catheter and endotracheal tube washings, and fungal cultures, depending on professional prescription.

Material

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.

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. 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.

Peripheral 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 ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL. 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 20–30mL 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.

Swabs/scrapes from respiratory tract, gastro-intestinal 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.

After sample taking place the probe into 1.5 mL plastic 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.

Faeces

Samples of faeces with mass (volume) 1-3 g (1-3 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.

Biopsy samples

Place a tissue biopsy sample in a 1.5 mL tube with transport medium for transportation and storage of biomaterial. Close the tube and mark it.

Washings from parts of intravenous catheter

Cut with sterile scissors 5-10 mm of a catheter tip and place it into a 1.5 ml tube of Eppendorf type. Close the tube and mark it.

Swabs 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 plastic 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

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 mL tube with 500 µL of sterile saline. 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 -18 °C to -20 °C no more than one week
- at -70 °C – 6 months

 Only one freezing-unfreezing of the material is allowed.

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

Swabs/scrapes from respiratory tract, gastro-intestinal 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.

Biopsy samples, parts of intravenous catheters, swabs and washings from endotracheal tubes, bacterial cultures

Biopsy samples, parts of intravenous catheters, swabs 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 -18 °C to -20 °C no more than one week;
- at -70 °C – 6 months.

 Only one freezing-unfreezing of the material is allowed.

Sample preparation

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 container 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 at 100-200 x g for 20 min.
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 min.
8. 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 min, shake the container every 2-3 min.

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 min.
3. Remove the supernatant completely.
4. Add 1.0 mL of sterile buffered saline to the precipitate.
5. Centrifuge the tube at 16000 x g for 10 min.
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.

Swabs/scrapes from respiratory tract, gastro-intestinal and urogenital tracts, swabs from endotracheal tubes, bacterial cultures from liquid and solid media

1. Centrifuge the tube at 16000 x g for 10 min.
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.

Faeces - preparation of the suspension

1. Put approximately 0,1-0,2 g (mL) of faeces into the 1.5 mL tube with 1.0 mL of sterile buffered saline.
2. Vortex the tube for 5-10 sec.
3. Further processing of the suspension is carried out in accordance with the instruction for the DNA extraction kit from the corresponding biomaterial.

Biopsy samples

1. Centrifuge the tube at 16000 x g for 10 min.
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 min.
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 in the tube with a fragment of catheter.
2. Vortex the tube for 3-5 sec and spin down the drops for 1-3 sec on vortex.
3. Add in the tube 300 µL of lysis buffer from PREP-NA DNA/RNA Extraction Kit.
4. Vortex the tube for 3-5 sec and spin down the drops for 1-3 sec on vortex.
5. Termostate the tube on 65 °C for 15 min.
6. Spin down the drops for 1-3 sec on vortex 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 PREP-NA DNA/RNA Extraction Kit 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.

Preparation and PCR procedure

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

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

2. Vortex the tube with Taq-polymerase solution for 3-5 seconds, then spin briefly for 1-3 seconds on vortex.
3. Add 10 µl of Taq-polymerase solution into each tube of the strip. Avoid paraffin layer break.
4. Add one drop (~20 µl) of mineral oil into each strip tube. Close the tubes tightly.
5. Vortex the tubes with DNA samples, positive control sample and negative control sample for 3-5 seconds, then spin down drops by centriguging on vortex-microcentrifuge for 1-3 seconds.

Open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next one to prevent contamination. Use filter tips.

6. Add 5 µl of DNA sample into corresponding strip tubes. Do not add DNA into the “C-“, “C+” strip tubes. Avoid paraffin layer break. Close the strips tightly.
7. Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into “C-“ tube and positive control (C+) into corresponding tube. Avoid paraffin layer break. Close the strips tightly.
8. Spin the strips briefly for 1-3 seconds on vortex.
9. Set the strips into the Real-time Thermal Cycler.

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 DTlite or DTprime 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 DTlite, DTprime and DT96 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

9. CONTROLS

The ***MycosoScreen*** **REAL-TIME PCR 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 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 RNA 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 and interpretation 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, swabs 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 ***MycosoScreen*** **REAL-TIME PCR 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 ***MycosoScreen REAL-TIME*** **PCR 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. For example, the sensitivity of the ***MycosoScreen REAL-TIME*** **PCR Detection Kit** for yeast culture is 50 copies/sample in case of extraction with PREP-NA DNA/RNA Extraction Kit (elution volume 50 µl).

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 PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact representative in EU or hotline@dna-technology.ru.

13. QUALITY CONTROL

"DNA-Technology Research&Production", LLC 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 by quality issues of ***MycosoScreen*** **REAL-TIME PCR Detection Kit**:

Technical support E-mail: hotline@dna-technology.ru,

www.dna-technology.com

Manufacturer: "DNA-Technology, Research & Production" LLC

Russia, 142281, Moscow Region, Protvino, 20 Zheleznodorozhnaya Street,

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

http://www.dna-technology.com

Authorized representative in EU:

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

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		Do not reuse
NON STERILE	Non-sterile		

REF R1-P023-S3/5EU VER 710.2021.08.10