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For professional use only

BacScreen OM REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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1. INTENDED USE

The **BacScreen OM REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications. The **BacScreen OM REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **BacScreen OM REAL-TIME PCR Detection Kit** is designed for DNA analysis of opportunistic bacteria from classes *Bacilli*, *Betaproteobacteria* and *Gammaproteobacteria* that cause nosocomial and community-acquired infections with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials (phlegm, urine, smears/scrapes of epithelial cells from respiratory tract, gastrointestinal tract and urogenital tract, feaces, aspirates, exudates) and bacterial cultures.

Indications for the use: symptoms of the infectious process.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **BacScreen OM REAL-TIME PCR Detection Kit.**

The **BacScreen OM 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 working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

Method: polymerase chain reaction (PCR) with detecting of the results in real time; 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 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 **BacScreen OM REAL-TIME PCR Detection Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains target-specific probes, bearing reporter fluorescent dyes 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 **BacScreen OM REAL-TIME PCR Detection Kit** contains PCR-mixes specific for DNA analysis of opportunistic bacteria and for determining auxiliary analytes: DNA of all bacteria (total bacterial load, TBL[1]), human DNA (sample intake control, SIC). PCR-mixes also contain an internal control (Internal Control, IC) which is intended to assess the quality of polymerase chain reaction in each tube (except tube № 16, see Table 1).

DNA probes for the detection of amplification products of bacterial DNA sites contain fluorescent dyes that are detected through channels Fam and Cy5; TBL – Fam, IC – Hex, SIC – Cy5.

The PCR-mix in tubes №1 and № 10 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.

The application of four fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube. Table 1 shows the detection channels of amplification products.

[1] The term previously used is total bacterial mass (TBM).

Table 1. Detection channels of amplification products

Type of strip	№ tube	Fam	Hex	Rox	Cy5	Color of buffer
«Strip №1»	1	TBL	IC	Marker	-	Blue
	2	*Streptococcus pyogenes*	IC	-	-	Colorless
	3	*Citrobacter freundii*	IC	-	*Citrobacter koseri*	
	4	*Burkholderia spp.*	IC	-	-	
	5	*Streptococcus pneumoniae*	IC	-	*Streptococcus spp.*	
	6	*Staphylococcus aureus*	IC	-	*Staphylococcus spp.*	
	7	*Klebsiella pneumoniae/ Klebsiella oxytoca*	IC	-	*Klebsiella pneumoniae*	
	8	*Acinetobacter spp.*	IC	-	-	
«Strip №2»	9	*Enterobacter cloacae*	IC	-	*Serratia marcescens*	
	10	*Stenotrophomonas maltophilia*	IC	Marker	*Haemophilus spp.*	Blue
	11	*Haemophilus influenzae*	IC	-	-	Colorless
	12	*Morganella morganii*	IC	-	*Enterobacteriales*	
	13	*Enterococcus spp.*	IC	-	SIC	
	14	*Escherichia coli*	IC	-	*Pseudomonas aeruginosa*	
	15	*Streptococcus agalactiae*	IC	-	*Proteus spp.*	
	16	*Achromobacter ruhlandii*	-	-	*Achromobacter xylosoxidans*	

The automatic analysis available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **BacScreen OM REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

3. CONTENT

The **BacScreen OM REAL-TIME PCR 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 **BacScreen OM REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P028-S3/6EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mixes, strip № 1	Colorless or blue transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	12 8-tube strips
Paraffin sealed PCR-mixes, strip № 2	Colorless or blue transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	12 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	2.0 mL (500 µL in each tube)	4 tubes
Mineral oil	Colorless transparent viscous oily liquid	4.0 mL (1.0 mL in each 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 **BacScreen OM REAL-TIME PCR Detection Kit** is intended for single use and designed for 12 tests (no more than 8 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, sterile single use flasks and sterile containers to collect clinical material;
- Sterile tubes containing transport medium ("DNA-Technology" made **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent for the transportation of the sample are recommended).

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-65 °C);
- 1.5 mL tubes;
- Tube rack for 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA PLUS** REF P-002/2EU **PREP-GS PLUS** REF P-003/2EU or **PREP-MB RAPID** REF P116-N/4EU, P116-A/8EU DNA/RNA extraction kits are recommended);
- Physiological saline solution 0.9% NaCl (Sterile);
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- 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;
- Refrigerator;
- Vortex mixer;
- Vortex rotor for 0.2 ml strips;
- PCR 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.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.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 **BacScreen OM REAL-TIME PCR 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 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-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 **BacScreen OM REAL-TIME PCR 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 **BacScreen OM REAL-TIME PCR 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. Wear powder-free surgical gloves. Wear 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 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-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 **BacScreen OM REAL-TIME PCR Detection Kit** is designed for DNA analysis of opportunistic bacteria from classes *Bacilli*, *Betaproteobacteria* and *Gammaproteobacteria* that cause nosocomial and community-acquired infections in DNA material obtained from phlegm, urine, smears /scrapes of epithelial cells from respiratory tract, gastrointestinal tract and urogenital tract, feaces, aspirates, exudates and bacterial cultures.

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 amplification.

PCR inhibitors are the following endogenous and exogenous interfering substances: the presence of hemoglobin, bilirubin, cholesterol, mucus (mucin) and drugs (pinosol, chlorhexidine bigluconate, indomethacin suppositories, rhinofluimucil, octenicept) in the DNA sample, 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 concentrations of interfering substances at which PCR inhibition was not observed are shown in the table below.

Type of biomaterial	Interfering substance (IS)	Concentration IS in the DNA sample
endogenous substances		
Feaces, urine, smears/scrape of epithelial cells from gastrointestinal tract	bilirubin	684 µmoL/L
Feaces, urine, smears/scrapes of epithelial cells from gastrointestinal tract	cholesterol	13 mmoL/L
Phlegm, urine, smears/scrapes of epithelial cells from respiratory tract, gastrointestinal tract and urogenital tract, feaces, aspirates, exudates	hemoglobin	0.35 mg/mL
Phlegm, smears/scrapes of epithelial cells from respiratory tract and urogenital tract, aspirates	mucus (mucin)	20%
exogenous substances		
Phlegm, smears/scrapes of epithelial cells from respiratory tract	pinosol	2.0%
Phlegm, smears/scrapes of epithelial cells from respiratory tract and urogenital tract, exudates	chlorhexidine bigluconate	5.0%
Feaces, smears/scrapes of epithelial cells from gastrointestinal tract	indomethacin suppositories	5.0%
Phlegm, smears/scrapes of epithelial cells from respiratory tract, aspirates	rhinofluimucil	5.0%
Phlegm, smears/scrapes of epithelial cells from respiratory tract and urogenital tract, aspirates, exudates	octenicept	2.0%
Phlegm, urine, smears/scrapes of epithelial cells from respiratory tract, gastrointestinal tract and urogenital tract, feaces, aspirates, exudates, bacterial cultures	isopropyl alcohol	10%
Phlegm, urine, smears/scrapes of epithelial cells from respiratory tract, gastrointestinal tract and urogenital tract, feaces, aspirates, exudates, bacterial cultures	methyl acetate	10%

Sample collection

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

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 and 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 special sterile dry flask by a single-use filtered pipette tip or single-use shovel. After sample collection the flask is tightly closed and marked.

Aspirates

Sample taking is made in single-use 50 mL tubes with screwing caps.

After sample collection, close the tube tightly and mark it.

Exudates

Order of taking:

1. Open the tube with a transport medium.

2. After sample taking put the swab into the tube with transport medium and rinse it thoroughly. Avoid spraying of solution.

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

4. Close the tube tightly and mark it.

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 1.5-2.0 mL tube with 500 µL of sterile physiological saline solution.

Close the tube tightly and mark it.

Transportation and storage of the samples

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, gastrointestinal and urogenital tracts, exudates

Smears/scrapes from respiratory tract, gastrointestinal and urogenital tract, exudates must be transported and stored according to the instructions for DNA extraction kits (**PREP-NA PLUS, PREP-GS PLUS**, **PREP-MB RAPID**).

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.

Aspirates, bacterial cultures

Aspirates, 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 by the **PREP-NA PLUS, PREP-GS PLUS** and **PREP-MB RAPID** kits. 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.

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 37.0 °C for 18–24 hours, then neutralize with 1M HCl (down to pH 6.8–7.4).
4. Centrifuge 1.5 mL tube at RCF(g) 900 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 RCF(g) 16000 for 10 minutes.
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 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 RCF(g) 16000 for 10 minutes.
3. Remove the supernatant completely.
4. Add 1.0 mL of sterile physiological saline solution to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 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, exudates, aspirates, bacterial cultures from liquid and solid media

1. Centrifuge the tube at RCF(g) 16000 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.

Faeces

1. Put approximately 250 mg (µL) of faeces into the 1.5 mL tube with 1.0 mL of sterile physiological saline solution.
2. Vortex the tube for 5-10 seconds.
3. Centrifuge the tube at RCF(g) 900 for 2-3 minutes.
4. Transfer 800–1000 µL liquid material to 1.5 mL plastic tube.
5. Centrifuge the tube at RCF(g) 16000 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.

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. The quality control of the extraction is carried out by the system of internal control (IC).

PREP-NA PLUS, **PREP-GS PLUS** and **PREP-MB RAPID** DNA/RNA extraction kits are 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 two strips with paraffin sealed PCR-mix (one Strip № 1 and one Strip № 2, 16 tubes total) for each test sample, for positive control (C+) and for negative control (C-).

Example: to test 2 samples, mark 8 strips - 4 strips for the samples, 2 strips for "C+" and 2 strips for "C-". See Table 3 for reference.

Table 3. Example of strip marking for PCR procedure

Samples	Ordinal number of strip	Type of strip
Sample A	1	Strip № 1
	2	Strip № 2
Sample B	3	Strip № 1
	4	Strip № 2
C-	5	Strip № 1
	6	Strip № 2
C+	7	Strip № 1
	8	Strip № 2

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

8.3 Add 10 µL of Taq-polymerase solution into each strip tube. 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.

In case of using **PREP-GS PLUS Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 3-5 seconds in a vortex mixer.

In case of using **PREP-MB RAPID Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 3-5 seconds in a vortex mixer.

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 strips. 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 strips. Avoid paraffin layer break.

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

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

8.10 Launch the operating software for DT instrument[2]. Add corresponding test[3], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the strips in the thermal unit (see 8.9) and run PCR. See Table 4.

Table 4. The PCR program for DTlite and DTprime 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[1]		…	Holding		Holding
√ - optical measurement [1] – holding at 25°C is allowed						

9. CONTROLS

The **BacScreen OM REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the bacterial and human genomes. 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 absent 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.

[2] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[3] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

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. 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) on three channels and decimal logarithms of concentrations (Lg) on two channels (excluding Hex).

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 of Strip № 1 and the second tube of Strip 2 are marked by a blue buffer) and correct identifiers of tubes in the protocol.

Analysis and interpretation of PCR results for **BacScreen OM REAL-TIME PCR Detection Kit**:

1. After the end of PCR the program, the software displays “+” or “-“ in the table in the “Result” column and makes a conclusion based on results of the test.
2. After the end of amplification, the software automatically calculates logarithms of concentrations from Cp data, which are indicated in the line with the name of the corresponding microorganism and TBL. The logarithms are displayed in the column with the corresponding gene or TBL. Based on the results of automatic comparison of logarithm values, the interval of the percentage of each microorganism from the total bacterial load in the DNA preparation is estimated, which is indicated in the report, in the “Result” column, in brackets after specifying the logarithm values.
3. In case of logarithm value of TBL more than 7.0, it is recommended to dilute DNA sample in 10-100 times and run PCR one more time to achieve more precise semiquantitative estimation.
4. Principles of automatic interpretation of results:
    - 4.1. If the obtained logarithm value corresponds to quantity of target less than 10 copies per amplification tube, the result is interpreted as negative and displayed in the "Analysis of optical measurements " column as "-", and in the specific report as “not detected”.
    - 4.2. The coefficient of efficiency is calculated automatically for each reaction. If the coefficient values exceed the acceptable limits, the reaction result is interpreted as “Invalid” (invalid result). In this case, in the "CE" column will indicate "-". This can be due to errors in amplification technology or sample preparation, in this case repeating of PCR amplification, or reextraction of DNA, or resampling is needed (performed sequentially).
    - 4.3. If the logarithm value of TBL is not specified while the logarithm values for the detected microorganisms are specified, then it is not possible to determine the percentage of microorganisms. This result is interpreted as “Invalid” (invalid result). This can be due to errors in amplification technology or sample preparation, in this case repeating of PCR amplification, or reextraction of DNA, or resampling is needed (performed sequentially).
    - 4.4. The Lg value for each microorganism must be less than Lg TBL + 0.5. If the values do not meet this requirement, then the determination of the percentage of microorganisms is not correct. This result is interpreted as “Invalid” (invalid result). This can be due to errors in amplification technology or sample preparation, in this case repeating of PCR amplification, or reextraction of DNA, or resampling is needed (performed sequentially).
    - 4.5. The Lg value for each microorganism must be less than Lg+0.5 for the higher taxonomic level.

Example: The Lg value for bacterial species Streptococcus pneumoniae must be less than the Lg+0.5 for

genus Streptococcus spp., to which this type of bacteria belongs.

If the values do not meet this requirement, then the determination of the percentage of microorganisms is not correct. This result is interpreted as “Invalid” (invalid result). This can be due to errors in amplification technology or sample preparation, in this case repeating of PCR amplification, or reextraction of DNA, or resampling is needed (performed sequentially).

4.6. In case of negative results obtained in one tube simultaneously for IC and for specific products registered on three detection channels (excluding the tube without IC, № 16 Strip № 2), in the "Result" column will be indicated “Invalid”. The result for this sample is not correct due to errors in amplification technology or sample preparation, in this case repeating of PCR amplification, or reextraction of DNA, or resampling is needed (performed sequentially).

The general principles of interpreting results are shown in Table A.1 of Annex A.

The decision on the etiological significance of the identified microorganism should be made on the basis of a comprehensive study in dynamics and in comparison with clinical and epidemiological data.

5. To access the quality of preanalytic stage and sample intake containing human cells, the parameter SIC (tube № 13, Strip № 2) is used. If there are no specific positive results in all tubes and the value of Lg SIC is less than 2.5, the negative result is considered as insufficient amount of biomaterial. In this case repeating of PCR amplification, or reextraction of DNA, or resampling is needed (performed sequentially). If the analyzing biomaterial does not contain human DNA or the specific nature of the test material predisposes to its trace amount (for example, pure bacterial cultures or hemocultures), the SIC value is not taking into account.

6. For positive and negative control samples the results must correspond to those from the Table 5. In the protocol for positive control sample the calculated logarithms are not compared with each other, as it is in case of clinical samples, and this situation is not programmatically interpreted as “Invalid” as described in paragraphs 3-5.

7. If results for negative control sample differ from those in the Table 5, the results of the whole series are considered invalid. In this case decontamination is required.

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

Detection channel				**Result**	**Interpretation of the result**
Fam	**Hex**	**Rox**	**Cy5**		
Positive control sample					
Cp is specified (for all tubes)	Is not considered	-	Cp is specified (for tubes № 3, 5-7, 9, 10, 12-16)	+	Positive result Results of the whole series are valid
Negative control sample					
Lg value TBL is not specified or ≤3.5; for others Lg are not specified	Cp is specified and <35* (except tube №16 which does not contain IC)	-	Lg value is not specified	-	The result is "less than 3.5 Lg" for TBL or negative Results of the whole series are valid
* other results are programmatically interpreted as an invalid result (“Invalid”) and may be associated with errors in amplification technology, in this case repeating of PCR amplification is required.					

11. SPECIFICATIONS

a. The analytical **specificity** of the **BacScreen OM 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 with DNA of the detected bacteria are to be registered positive for specific product through the declared detection channels.

The samples free of DNA of the detected bacteria are to be registered negative for specific product through the declared detection channels.

For each test in the kit, there are not cross non-specific results with all other tests from the kit.

There are not non-specific positive results of amplification of DNA sample in the study of high concentrations of DNA (at least $2.0x10^3$ copies per amplification tube) of conditionally pathogenic and other microorganisms that cause infectious diseases, and/or normally present in the loci of biomaterial sampling: *S. pyogenes, C. freundii, C. koseri, Burkholderia spp., S. pneumoniae, Streptococcus spp., S. aureus Staphylococcus spp., K. pneumoniae, K. oxytoca, Acinetobacter spp., E. cloacae, S. marcescens, S. maltophilia, Haemophilus spp., H. influenzae, M. morganii, Enterococcus spp., Campylobacter spp., Candida spp., Chlamydophila pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Salmonella spp.*, as well as human DNA.

b. Analytical **sensitivity** of the **BacScreen OM REAL-TIME PCR Detection Kit** is 10 copies of DNA per amplification tube ($2.0x10^3$ copies/mL DNA sample). Sensitivity is determined by the analysis of serial dilutions of the laboratory control sample (LCS).

Sensitivity depends the extraction kit used for DNA extraction and the final elution volume (dilution) of the extracted DNA. For example, the sensitivity of the **BacScreen OM REAL-TIME PCR Detection Kit** for bacterial culture is 600 copies/sample in case of extraction with **PREP-NA PLUS DNA/RNA Extraction Kit** (elution volume 300 µL).

c. Diagnostic characteristics

Type of biomaterial	Number of samples	Diagnostic sensitivity	Diagnostic specificity
Phlegm	12*	100% (79.41 - 100)	100% (98.71 - 100)
Urine	63	100% (97.14 - 100)	100% (99.75 - 100)
Smears/scrapes from respiratory tract	18	100% (90.51 - 100)	100% (99.11 - 100)
Smears /scrapes of epithelial cells from gastrointestinal tract	9	100% (86.77 - 100)	100% (98.16 - 100)
Smears /scrapes from urogenital tract	7	100% (81.47 - 100)	100% (98.16 - 100)
Faeces	6	100% (81.47- 100)	100% (97.24 - 100)
Aspirates	9	100% (82.35- 100)	100% (98.23 - 100)
Exudates	8	100% (80.49 - 100)	100% (98.00 - 100)
Total	**132**	**100% (98.68 - 100)**	**100% (99.88 - 100)**
Bacterial cultures	**46**	**100% (95.07 – 100)**	**100% (99.66 – 100)**
* - including 5 synthetic samples			

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 our customer service department regarding quality issues with the kit:

Phone: +7(495)640.16.93

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com/support

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 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 **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista GLA Van/Mec REAL-TIME PCR Detection Kit.**

Technical support:

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14. KEY TO SYMBOLS

	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests		Catalogue number
	Use-by date		Manufacturer
	Batch code		Keep away from sunlight
	Caution		Version
	Do not reuse		Positive control
	Authorized representative in the European Community		Non-sterile

Annex A

Results interpretation principles

Detection, data analysis and logarithm of pathogen DNA concentrations calculation are made by software automatically. In this supplementary results interpretation principles are described.

Table A.1

Detection channel				Result	Interpretation
Fam	Hex	Rox	Cy5		
Samples					
Lg value is specified	Is not considered	-	Lg value is specified	+	DNA of identifying bacteria is detected
Lg value is not specified	Cp is specified (for the same tubes as on Fam\Cy5 channels), tube № 16 – does not contain IC	-	Lg value is not specified	-	DNA of identifying bacteria is not detected
Cp is not specified	Cp is not specified (for the same tubes as on Fam\Cy5), tube № 16 – does not contain IC	-	Cp is not specified	invalid	Invalid result