DNA-TECHNOLOGY

INFECTIONS

BACSCREEN OM

REAL-TIME PCR DETECTION KIT FOR DETECTION OF DNA OF OPPORTUNISTIC BACTERIA OF THE CLASSES BACILLI, BETAPROTEOBACTERIA AND GAMMAPROTEOBACTERIA, CAUSING NOSOCOMIAL AND COMMUNITY-ACQUIRED INFECTIONS

CLINICAL SIGNIFICANCE

Opportunistic microorganisms (OM) can become the reason of a developing infectious process under several conditions like chronic diseases, traumas, specific medical therapy, hospitalizations, pregnancy and childbirth, young or old age.

Medical centers have special hospital flora with certain types of microorganisms (OM) that are potential infectious agents. They can be transmitted from other patients or from medical personnel and cause nosocomial infections, which often lead to severe disease and prolonged hospitalization [1].

Infections, caused by OM, can vary from purulent-inflammatory skin diseases to pneumonia, necrotizing fasciitis and meningitis. OM can also cause community-acquired infections, such as upper and lower respiratory and urinary tract infections, skin and soft tissue infections, etc. Such infections are especially dangerous for patients with chronic diseases (diabetes mellitus, cystic fibrosis, alcoholism, etc.) [2].

It is impossible to determine the pathogen accurately without laboratory tests, because OM cause infections similar in clinical manifestations. Differential diagnosis is necessary to select the therapy correctly. It is important to consider that OM can get new features, such as antibiotic resistance, the ability of cell surface adhesion and biofilm formation [3].

Several representatives of OM have been united in a separate group by Infectious Diseases Society of America (IDSA). ESKAPE pathogens are nosocomial infections causative agents that can «avoid» the bactericidal effects of antibiotics (ESKAPE: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.*) [5].

Opportunistic bacteria of classes *Bacilli*, *Betaproteobacteria* and *Gammaproteobacteria* and their clinical significance

№	OM	Clinical significance
1	*Achromobacter ruhlandii*	The most clinically significant bacteria for patients with cystic fibrosis[4]
2	*Achromobacter xylosoxidans*	
3	*Acinetobacter spp.*	Common causative agents of severe nosocomial infections. ESKAPE pathogen *A. baumannii* is often multidrug-resistant [5, 6]
4	*Burkholderia spp.*	Clinically significant for patients with cystic fibrosis [4]
5	*Citrobacter freundii*	Often acquires antibiotics multidrug resistance [7]
6	*Citrobacter koseri*	Often causes infections in newborns and in immunocompromised patients [8]
7	*Enterobacter cloacae*	Has resistance to antibiotics. ESKAPE pathogen [5, 8]
8	*Enterobacteriales*	Includes many clinically significant species (*Citrobacter spp, E. coli, Klebsiella spp*, etc.)
9	*Enterococcus spp.*	*E. faecium* (80–90% of all enterococci isolated from humans) causes nosocomial infection, can be often resistant to antibiotics; ESKAPE pathogen [5, 9]. *E. faecalis* (5–10%) is a causative agent of community-acquired infections
10	*Escherichia coli*	Dominant causative agent of nosocomial infections, including those with multiple antibiotic resistance [10]
11	*Haemophilus spp.*	Includes many clinically significant species (*H. influenzae, H. parainfluenzae, H. haemolyticus etc.*). *H. parainfluenzae* can cause up to 4% of community-acquired pneumonia [11]
12	*Haemophilus influenzae*	One of the main causative agents of lung infection in patients with cystic fibrosis [4]. More than 90% of invasive *H. influenzae* infections occur to children younger than 5 years (mostly newborns)
13	*Klebsiella pneumoniae*	Often leads to infectious pathologies and can be lethal to immunosuppressed patients. ESKAPE pathogen [5, 12]
14	*Klebsiella pneumoniae / Klebsiella oxytoca*	Can have antibiotic drug resistance [13]
15	*Morganella morganii*	Causes infections of postoperative wounds and urinary tract. Some strains are resistant to multiple antibiotics. Can cause various infectious diseases with high mortality [19]

№	OM	Clinical significance
16	*Serratia marcescens*	Causes outbreaks of nosocomial infections. Some isolates are resistant to extended-spectrum β-lactamase (ESBLs) or imipenem [14]
17	*Staphylococcus spp.*	Especially dangerous for immunocompromised patients, patients with chronic diseases and prolonged hospitalized people. It is often antibiotic resistant [15]
18	*Staphylococcus aureus*	May lead to the development of bacteremia, endocarditis; osteoarticular and pleuropulmonary infections, as well as to infections of the skin and soft tissues. Especially clinically significant for patients with cystic fibrosis. ESKAPE pathogen [4, 5, 16]
19	*Stenotrophomonas maltophilia*	Often has multidrug resistance. Clinically significant for patients with cystic fibrosis. [4, 17]
20	*Streptococcus spp.*	Can cause infections of various localization and severity, including invasive [18]
21	*Streptococcus agalactiae*	May cause meningitis, bacteremia in newborns; postpartum infections in parturient women [18]
22	*Streptococcus pneumoniae*	One of the main causative agents of pneumonia in the world, affects immunocompromised patients [18]
23	*Streptococcus pyogenes*	Causes both mild and severe infections, including necrotizing fasciitis, streptococcal toxic shock syndrome and bacteremia [18]
24	*Proteus spp.*	*P. mirabilis, P. vulgaris* cause urinary tract infections, wound infections and meningitis. *P. mirabilis* is multidrug resistant [20]
25	*Pseudomonas aeruginosa*	Clinically significant for patients with cystic fibrosis. ESKAPE pathogen [4, 5]

Microscopic, cultural, serological and molecular genetics methods are used to diagnose bacterial infections

- Light microscopy of Gram-stained smears allows to determine the microflora and the ratios of its components
- Bacteriological culture is used to isolate microorganisms' pure cultures and identify the species. It is possible to determine the sensitivity of bacteria to antibiotics in combination with this method
- Serological methods detect antibodies or antigens in patient's blood. The method has timing requirements to take the sample and lacks of specificity.
- Molecular genetics methods are designed to detect specific fragments of microorganisms and identify the pathogen. Real-time polymerase chain reaction (PCR) is a fast and highly sensitive method

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.

TESTING IS RECOMMENDED

for symptoms
of a bacterial infection.

BIOMATERIAL:

- human biological material:
  - sputum
  - urine
  - swabs / scrapings from the respiratory tract, gastrointestinal and urogenital tracts
  - feces
  - aspirates
  - exudates
- bacterial cultures

ADVANTAGES OF PCR DIAGNOSIS FOR BACTERIAL INFECTIONS COMPARED TO OTHER DIAGNOSTIC METHODS

- Identification of microorganisms **to a given taxonomic order**: accurate identification of the causative agent of the disease
- Identification of **difficult-to-culture** microorganisms
- **Fast**
- **High analytical sensitivity**
- Does not require manipulations with **live bacterial cultures**
- Possibility to study a **wide range of biomaterials**

SPECIAL FEATURES OF THE KIT

- **A complex test** that allows to identify a wide range of bacteria of three classes and determine the ratio of each pathogen in DNA sample
- **Multiplex format** — Several targets are detected simultaneously in one tube
- **Sample intake control (SIC)** in case of using human biomaterial
- **Internal control** — assessment of the quality of the progress of the polymerase chain reaction
- **Automatic generation of the results form** when using the recommended Real-time PCR instruments of the DT series and RealTime_PCR software
- **Availability of preset templates with test parameters, which automatically set the necessary settings** and calculate the results

It is recommended to use the **BacResista GLA, BacResista GLA Van/Mec Real-time PCR kits** for detection of resistance genes to glycopeptide and beta-lactam antibiotics in bacteria in conjunction with with BacScreen OM PCR kit.

KIT SPECIFICATIONS

Composition of the kit:

- Paraffin sealed PCR-mixes (strips N1, N2)
- Taq-polymerase solution
- mineral oil
- positive control
- strip's caps

The kit is designed for 12 tests, including positive and negative controls.

Hands-on time
(without sample preparation): from 1.5 hour.

DETECTION CHANNELS OF AMPLIFICATION PRODUCTS

strip type	Strip tube №	Fam	Hex	Rox	Cy 5
Strip №1	1	TBM	IC	Marker	–
	2	*Streptococcus pyogenes*	IC	–	–
	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 type	Strip tube №	Fam	Hex	Rox	Cy 5
Strip №2	9	*Enterobacter cloacae*	IC	–	*Serratia marcescens*
	10	*Stenotrophomonas maltophilia*	IC	Marker	*Haemophilus spp.*
	11	*Haemophilus influenzae*	IC	–	–
	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*

Analytical sensitivity	10 copies of nucleic acid per amplification tube (2.0x10^3 copies/mL DNA sample)

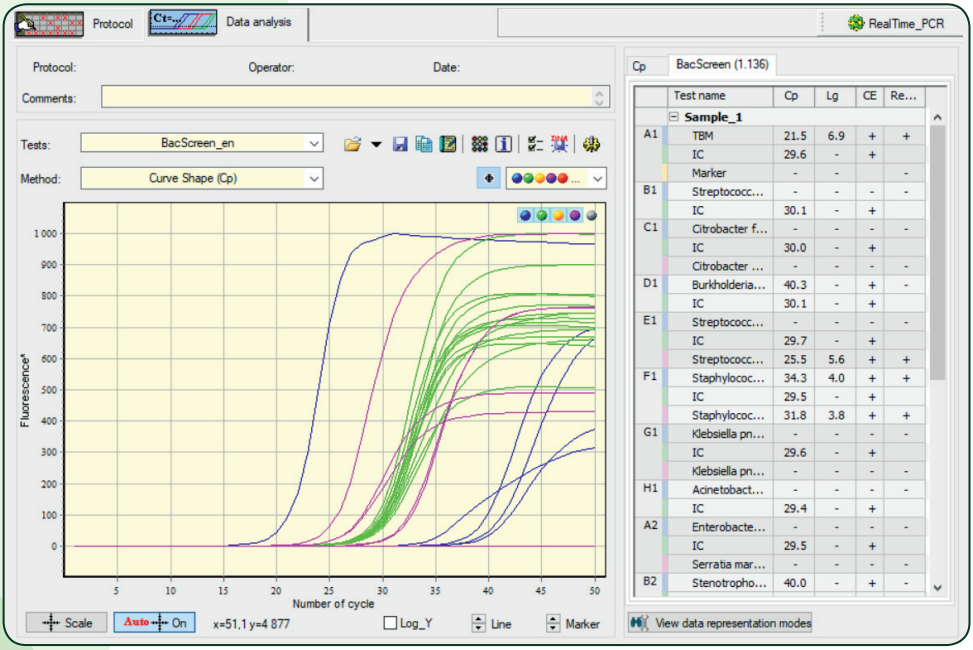
RECOMMENDED MATERIALS AND EQUIPMENT

Transport medium	DNA extraction kits	Real-time PCR instruments
▪ STOR-M ▪ STOR-F	▪ PREP-NA PLUS ▪ PREP-GS PLUS ▪ PREP-MB RAPID	▪ DTprime ▪ DTlite
produced by DNA-Technology		

SOFTWARE

- Real-Time PCR Software

Registration and interpretation of the reaction results are carried out automatically using the Real-Time PCR software for Real-time PCR instruments of the “DT” series manufactured by “DNA-Technology R&P”, LLC.



An example of the result of a PCR assay using a real-time PCR instrument of the DT series and related software: analysis of optical measurements

AN EXAMPLE OF THE RESULT FORM

BacScreen OM

Data:
Tube number:
Patient:
Sex:
Age:
Physician:
Comment:

Logotype

Information about laboratory

Sample ID:

№	Test name	Result
1	TBM	DETECTED (6.9 lg)
2	*Achromobacter ruhlandii*	not detected
3	*Achromobacter xylosoxidans*	not detected
4	*Acinetobacter spp.*	not detected
5	*Burkholderia spp.*	not detected
6	*Enterobacteriales*	DETECTED (3.5 lg, <0.1%)
6.1	*Citrobacter freundii*	not detected
6.2	*Citrobacter koseri*	not detected
6.3	*Enterobacter cloacae*	not detected
6.4	*Escherichia coli*	not detected
6.5	*Klebsiella pneumoniae + Klebsiella oxytoca*	not detected
6.5.1	*Klebsiella pneumoniae*	not detected
6.6	*Morganella morganii*	not detected
6.7	*Proteus spp.*	not detected
6.8	*Serratia marcescens*	not detected
7	*Enterococcus spp.*	not detected
8	*Haemophilus spp.*	DETECTED (5.3 lg, 2.0-2.4%)
8.1	*Haemophilus influenzae*	not detected
9	*Pseudomonas aeruginosa*	not detected
10	*Staphylococcus spp.*	DETECTED (3.8 lg, <0.1%)
10.1	*Staphylococcus aureus*	DETECTED (4.0 lg, 0.1-0.1%)
11	*Stenotrophomonas maltophilia*	not detected
12	*Streptococcus spp.*	DETECTED (5.6 lg, 4-5%)
12.1	*Streptococcus agalactiae*	not detected
12.2	*Streptococcus pneumoniae*	not detected
12.3	*Streptococcus pyogenes*	not detected

Results Form of **BacScreen OM** PCR analysis was obtained using real-time PCR instrument of the DT series and related software.

TRANSPORT AND STORAGE CONDITIONS

The kit must be stored at temperatures from 2 °C to 8 °C during the storage period.

Transportation is allowed in thermoboxes with ice packs by all types of roofed transport at temperatures from 2 to 25 °C but no more than 5 days.

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