DNA-TECHNOLOGY

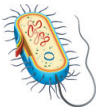
ANTIBIOTIC RESISTANCE

BACRESISTA GLA, BACRESISTA GLA VAN/MEC

REAL-TIME PCR KITS FOR DETECTION OF THE GENES RESPONSIBLE FOR RESISTANCE TO GLYCOPEPTIDE AND BETA-LACTAM ANTIBIOTICS IN BACTERIA

CLINICAL SIGNIFICANCE

The increasing prevalence of microorganisms' strains resistant to antibacterial drugs (ABDs) makes it more difficult to treat patients with bacterial infections [1]. ABDs' resistance hinders effective therapy and contributes to the emergence of chronic and recurrent infections. Diseases caused by antibiotic resistant strains are characterized by a long duration, necessity of hospitalization and a bad prognosis for patients [2].



The microorganisms' drug resistance can be natural and acquired, but its formation is genetically determined in all cases. Antibiotic resistance genes are often localized on mobile genetic elements and are capable of spreading rapidly within the same species and between different bacteria species [3, 4]. Excessive and unreasonable prescription of ABD, self-medication and the usage of ABD in agriculture facilitate the intensification of this process.

Phenotypic (microbiological) and molecular genetics methods, including polymerase chain reaction (PCR), are used to detect resistance to ABD.

PCR results correlate with the results obtained by disk diffusion method and the serial dilution method [5, 6]. Meanwhile PCR assay does not require manipulation with live bacterial cultures. It prevents the spread and circulation of microorganisms inside medical diagnostic and laboratory institutions. PCR diagnostics can significantly decrease the time needed to get the results. It could be critically important for severe infections' treatment because the effect of etiotropic therapy largely depends on the timing of the appropriate antibiotics prescription [7].

PCR can be used to determine the antibiotic resistance genes in conjunction with phenotypic methods to prescribe etiological therapy even before the pathogen's differentiation in pure culture [8]. PCR diagnostics of antibiotic resistance is also recommended when the result of the cultural method has unclear growth suppression zone [9]. Also it is possible to combine the PCR assay with microbiological culture methods to determine the antibiotic sensitivity.

Determination of antibiotic resistance genes is an important additional clinical information for the doctor. It cannot be obtained by the microbiological method. PCR diagnostics is a direct method that allows to detect the antibiotic resistance genes in difficult-to-culture microorganisms. The result of the PCR study is not affected by the storage conditions of ABD and culture media.

THE BACRESISTA KIT

is designed for DNA analysis of bacteria resistant to glycopeptide (G) and beta-lactam (L) antibiotics (A) in DNA material obtained from biological samples and bacterial cultures by with an aid of Polymerase Chain Reaction (PCR) method.

The «BacResista» set is available in the following versions:

- «BacResista GLA» – determines a wide range of resistance genes to glycopeptide and beta-lactam antibiotics;
- «BacResista GLA Van/Mec» is a truncated version of BacResista GLA.

Resistance gene	Antibacterial drugs	BacResista GLA	BacResista GLA Van/Mec
van A/B	vancomycin, teicoplanin	✓	✓
mec A	methicillin, oxacillin	✓	✓
tem	penicillins and cephalosporins	✓	
ctx-M-1		✓	
shv		✓	
oxa-40-like	carbapenems	✓	
oxa-48-like		✓	
oxa-23-like		✓	
oxa-51-like		✓	
imp		✓	
kpc		✓	
ges		✓	
ndm		✓	
vim		✓	
Bacteria group		Gram (+) and Gram (-)	Gram (+)

According to the results of «BacResista GLA» study it is possible to conduct a semi-quantitative assessment:

- determination of the proportion of resistant microorganisms from the total bacterial mass;
- determination of the linkage of resistance genes with each other.

THE ASSAY IS RECOMMENDED

for a quick assessment of the presence of bacterial resistance genes to glycopeptide and beta-lactam antibiotics in the biological sample.

MATERIAL UNDER STUDY:

- human biomaterial:
  - phlegm;
  - urine;
  - swabs / scrapings from the respiratory, gastrointestinal and urogenital tract;
  - feaces;
  - aspirates;
  - exudates;
- bacterial cultures.

ADVANTAGES OF PCR DIAGNOSTICS IN COMPARISON WITH PHENOTYPICAL ANALYSIS OF SUSCEPTIBILITY TO ABD

- **Additional express information** about the presence of antibiotic resistance genes in bacteria can help to reduce the time for choosing the optimal antibiotic therapy.
- The use of an instrumental PCR technique allows obtaining **reproducible results**, which do not depend on the features of the microbiological laboratory equipment.
- The possibility of detecting antibiotic resistance in **difficult-to-culture microorganisms**.
- Possibility to study a **wide range of biomaterials**.
- **High analytical sensitivity**.

SPECIAL FEATURES OF THE KIT

- Complex test that allows to identify **a wide range of resistance genes**.
- Possibility of use in conjunction with a **«BacScreen OM» PCR kit** for detection of DNA of opportunistic bacteria that cause nosocomial and community-acquired infections.
- In the version of «BacResista GLA» **a semi-quantitative analysis** is implemented.
- **Multiplex format** – several DNA targets are detected simultaneously in one tube.
- **Internal control** – assessment of PCR quality.
- **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.

KIT SPECIFICATIONS

Composition of the kit:

- paraffin sealed PCR-mixes (strips);
- Taq polymerase solution;
- mineral oil;
- positive control;
- strip's caps.

The «BacResista» GLA kit version is designed for 24 tests, including positive and negative controls.

The «BacResista» GLA Van/Mec kit version is designed for 48 tests, including positive and negative controls.

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

DETECTION CHANNELS OF AMPLIFICATION PRODUCTS:

strip tube №	Detection channel			
	Fam	Hex	Rox	Cy5
BacResista GLA				
1	*imp*	IC	—	—
2	TBL	IC	—	*oxa-51-like*
3	*ctx-M-1*	—	—	*tem*
4	*van A/B*	IC	—	*mec A*
5	*oxa-48-like*	IC	—	*oxa-40-like*
6	*vim*	IC	—	*kpc*
7	*oxa-23-like*	IC	—	*ndm*
8	*shv*	IC	Marker	*ges*
BacResista GLA Van/Mec				
	van A/B	IC	—	*mec A*

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

RECOMMENDED MATERIALS AND EQUIPMENT

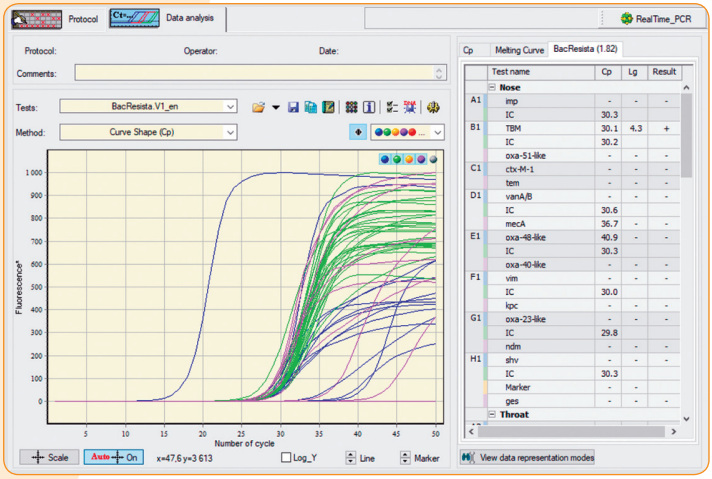
DNA extraction kits	Real time PCR instruments
▪ PREP-NA DNA/RNA Extraction Kit ▪ PREP-NA PLUS DNA/RNA Extraction Kit ▪ PREP-GS DNA Extraction Kit ▪ PREP-GS PLUS DNA Extraction Kit ▪ PREP-MB RAPID	▪ DTprime ▪ DTlite

produced by «DNA-Technology R&P», LLC

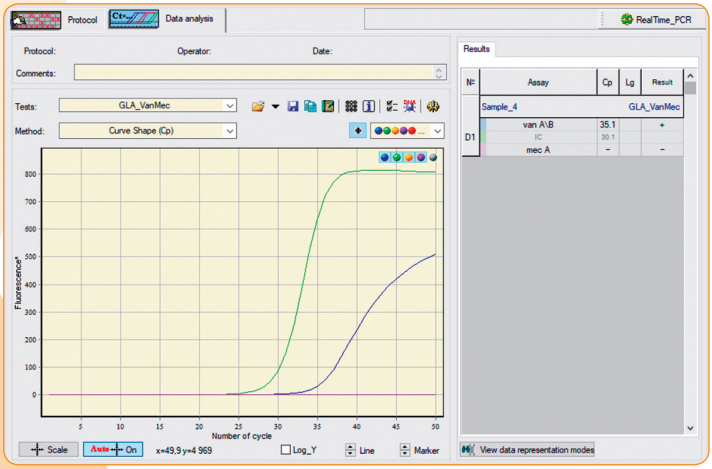
- RealTime_PCR Software

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.

A.



B.



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.
A — BacResista GLA, B — BacResista GLA Van/Mec

AN EXAMPLE OF THE RESULT FORM

A.

BacResista GLA

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

Sample ID:

logotype

information about laboratory

№	Test name	Result
1	*imp*	not detected
2	TBM	not detected
3	*oxa-51-like*	not detected
4	*ctx-M-1*	DETECTED (3.3 Lg)
5	*tem*	not detected
6	*van A/B*	not detected
7	*mec A*	not detected
8	*oxa-48-like*	not detected
9	*oxa-40-like*	not detected
10	*vim*	not detected
11	*kpc*	not detected
12	*oxa-23-like*	not detected
13	*ndm*	not detected
14	*shv*	not detected
15	*ges*	not detected

Conclusion:
Beta-lactam antibiotic resistance gene (s) is (are) found. Manifestations of resistance to penicillins, cephalosporins of the I-IV generation, monobactams in gram-negative bacteria are possible.

Study was carried out by:

Data:
Signatyre:

AN EXAMPLE OF THE RESULT FORM

B.

PCR result

Data:
Number of tube:
Patient name:
Sex:
Age:
Organization:
Clinician name:
Comments:

Sample ID:

logotype

information about laboratory

№	Name of research	Result
1	*van A/B*	detected
2	*mec A*	not detected

Study was carried out by:

Data:
Signatyre:

Results Form of BacResista PCR analysis was obtained using a detecting thermocycler real-time PCR instrument of the «DT» series and related software.
A — BacResista GLA, B — BacResista GLA Van/Mec

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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