DNA-TECHNOLOGY

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CE IVD

For professional use only

BacResista GLA REAL-TIME PCR Detection Kit

BacResista GLA Van/Mec REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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,

142281, Russia,

Moscow Region, Protvino,

Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Customer service department

E-mail: hotline@dna-technology.ru

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R1-P027-S3/4EU

R1-P027-23/4EU

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1. INTENDED USE

The **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista GLA Van/Mec REAL-TIME PCR Detection Kit** are intended for research and diagnostic applications. The **BacResista GLA REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista GLA Van/Mec REAL-TIME PCR Detection Kit** are 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 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: the need to study a possible antibiotic resistance in bacteria that caused infectious disease.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista GLA Van/Mec REAL-TIME PCR Detection Kit.**

The **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista GLA Van/Mec 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; qualitative and semi-quantitative multiplex analysis.

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 ***BacResista GLA*** and ***BacResista Gla Van/Mec*** **REAL-TIME PCR Detection Kits** are 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.

The **BacResista GLA REAL-TIME PCR Detection Kit** contains amplification mixtures specific for antibiotic resistance genes (van A\van B, mec A, imp, oxa-51-like, tem, ctx-M-1, oxa-40-like, oxa-48-like, kpc, ges, ndm, oxa-23-like, shv, vim) and for DNA of all bacteria (total bacterial mass, TBM).

The **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit** contains amplification mixtures specific for antibiotic resistance genes (van A\van B, mec A).

The PCR-mix (except tube №3 in the **BacResista GLA REAL-TIME PCR Detection Kit**) includes Internal control (IC), which is intended to assess the quality of polymerase chain reaction. DNA probes used for the detection of antibiotic resistance genes amplification products include fluorescent dyes Fam and Cy5. DNA

probe used for the detection of the internal control amplification product includes fluorescent dye Hex.

The PCR-mix in tube №8 in the **BacResista GLA REAL-TIME PCR Detection Kit** 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.

Table 1. Detection channels of amplification products

№ tube	Dye label/detection channel					**Color of the buffer**
	Fam	**Hex**	**Rox**	**Cy5**	**Cy5.5**	
BacResista GLA						
1	imp	IC**	-	-	-	Blue
2	TBM*	IC	-	oxa-51-like	-	Colorless
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	-	Colorless

*TBM – total bacterial mass, **IC –internal control

The automatic analysis is available on "DNA-Technology" made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista GLA Van/Mec 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 **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista GLA Van/Mec REAL-TIME PCR Detection Kit** contains paraffin sealed PCR-mix, Taq-polymerase solution, mineral oil and positive control. The detailed description of content is represented in Tables 2-4.

Table 2. The **BacResista GLA REAL-TIME PCR Detection Kit** content, package S (standard) for R1-P026-S3/5EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or blue transparent liquid under waxy white fraction	3840 µL (20 µL in each tube)	24 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	2000 µL (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		

Table 3. The **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit** content, package S (standard), strips for R1-P027-S3/4EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	960 µL (20 µL in each tube)	6 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	75 µL	1 tube
Strip's caps	6 8-caps		

Table 4. The **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit** content, package S (standard), tubes for R1-P027-23/4EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	960 µL (20 µL in each tube)	48 tubes
Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
Positive control	Colorless transparent liquid	75 µL	1 tube

All components are ready to use and do not require additional preparation for operation.

The **BacResista GLA 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).
The **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit** is intended for single use and designed for 48 tests (no more than 46 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 media or physiological saline solution for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** REF P-002/1EU, **PREP-NA PLUS** REF P-002/2EU, **PREP-GS** REF P-003/1EU, **PREP-GS PLUS** REF P-003/2EU or **PREP-MB RAPID** REF P116-N/4EU, P116-A/8EU extraction kits are recommended);
- High speed centrifuge (RCF(g) no less than 16000);
- Solid-state thermostat (temperature range 50-65 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- RNase and DNase free non-filtered pipette tips for aspirator with trap flask;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Vortex mixer;
- Vortex rotor for strips (using kits R1-P026-S3/5EU and R1-P027-S3/4EU);
- Refrigerator;
- PCR tube rack for 0.2 mL tubes or strips;
- 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 the **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista GLA Van/Mec 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 has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

It is allowed to transport the kit in thermobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

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 kit stored in under undue regime should not be used.

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

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 being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, 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. 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 **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista GLA Van/Mec REAL-TIME PCR Detection Kit** are designed for DNA analysis of bacteria resistant to glycopeptide (G) and beta-lactam (L) antibiotics (A) in DNA material obtained from phlegm, urine, smears/scrapes from respiratory tract, gastro-intestinal and urogenital tracts, faeces, 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 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.

Impurities contained in the biomaterial sample, such as mucus, blood, elements of tissue breakdown and inflammation, local medicines, including those that are contained in vaginal suppositories, talc, spermicide, etc. should be removed during the DNA extraction using sample preparation kits. To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose DNA extraction methods that allow to remove PCR inhibitors from the sample as much as possible. It is not recommended to use express methods of DNA extraction.

General requirements

PCR analysis refers to direct methods of laboratory research, therefore the collection of biological material must be carried out from the site of infection localization. Professional prescription is required to localize the place of sampling. The decision must be based on patient's complaints and clinical signs, and made by the physician in charge.

To interpret results successfully and robustly, a high quality of sample and appropriate conditions of storage, transport, and handling are required.

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

Sample collection

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

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, 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.

 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, gastro-intestinal 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 saline.

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, gastro-intestinal and urogenital tracts, exudates

Smears/scrapes from respiratory tract, gastro-intestinal and urogenital tract, exudates must be transported and stored according to the instructions for DNA extraction kits (**PREP-NA**, **PREP-NA PLUS, PREP-GS**, **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**, **PREP-NA PLUS, PREP-GS**, **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 5.0% 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 approximately 100 µL (precipitate+liquid fraction) in the tube.

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 buffered saline to the precipitate.
5. Centrifuge the tube at RCF(g) 16000 for 10 minutes.
6. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction) in the tube for DNA extraction with **PREP-GS** or **PREP-GS PLUS** or 100 µL for DNA extraction with **PREP-NA**, **PREP-NA PLUS** and **PREP-MB RAPID**.

Smears/scrapes from respiratory tract, gastro-intestinal and urogenital tracts, exudates, aspirates, bacterial cultures from liquid and solid media

1. Centrifuge the tube at 16000 x g for 10 minutes.
2. Remove the supernatant, leaving approximately 50 µL (precipitate+liquid fraction) in the tube for DNA extraction with **PREP-GS** or **PREP-GS PLUS** or 100 µL for DNA extraction with **PREP-NA**, **PREP-NA PLUS** and **PREP-MB RAPID**.

Faeces

1. Put approximately 250 mg (µL) of faeces into the 1.5 mL tube with 1.0 mL of sterile buffered saline.
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 approximately 100 µL (precipitate+liquid fraction) in the tube.

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. **PREP-NA**, **PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS** and **PREP-MB RAPID** DNA/RNA Extraction Kits are recommended. 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.

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.

In case of using tubes in strips, 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 For **BacResista GLA REAL-TIME PCR Detection Kit:** 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 5 for reference.

8.2 For **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit:** Mark one strip tube (or single tube) 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 tubes - 2 tubes for the samples, 1 tube for "C-" and 1 tube for "C+". See Table 5 for reference.

Table 5. Example of strip or tube marking for PCR procedure

Samples	**BacResista GLA**	**BacResista Gla Van/Mec**
Sample 1	Strip 1	Tube 1
Sample 2	Strip 2	Tube 2
C-	Strip 3	Tube 3
C+	Strip 4	Tube 4

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

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

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

8.6 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** and **PREP-GS PLUS** extraction kits. 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 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. Close the tubes/strips tightly. Use filter tips.

8.7 Add 5.0 µL of DNA sample into corresponding strips/tubes. Do not add DNA into the "C-", "C+" strip tubes. Avoid paraffin layer break.

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

8.9 Spin the strips/tubes for 1-3 seconds.

8.10 Set the strips/tubes into the Real-time Thermal Cycler.

8.11 Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the strips/tubes in the thermal unit (see 8.10) and run PCR. See Table 6.

Table 6. 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	…	…	Holding		Holding
√ - optical measurement						

9. CONTROLS

The **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit** contain positive control sample. Positive control is a cloned part of the bacterial genome. It is produced with genetic engineering techniques and characterized by automatic sequencing. The PCR-mix from the kit includes the internal control. IC is an artificial plasmid intended to assess the quality of PCR performance. To reveal possible contamination a negative control is required.

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

The test result is considered valid when:

- the exponential growth of the fluorescence level for the specific product is present, in this case the internal control is not taken into account;

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

- 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.

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.

Analysis and interpretation of PCR results for **BacResista GLA REAL-TIME PCR Detection Kit**:

1. After the end of amplification the software automatically calculates logarithms of concentrations from Cp data. The logarithms are displayed in the column with the corresponding gene or TBM. Comparing the logarithms of concentrations, the corresponding semiquantitative estimation can be made:

- the part of resistant microorganisms from total bacterial mass;
- the correlation of antibiotic resistance genes with one another.

2. Logarithm values less than 3.0 are not calculated, but Cp for those tubes is indicated. Those results are interpreted as negative and are displayed in the “Analysis of optical measurements” column as “-” and in the conclusion as “not detected”.
3. In case of logarithm value of TBM 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. If the logarithm value of TBM is not specified while the logarithm values for resistance genes are specified, semiquantitative estimation is not correct, but the results can be recorded as qualitative. Thus it is interpreted as positive result and displayed in the “Analysis of optical measurements” column as “+” and in the conclusion as “detected”. This can be due to errors in amplification technology, in this case the repeat of PCR run is required to make semiquantitative estimation possible.
5. The Lg value for genes of antibiotic resistance must be less than Lg of TBM+0.5. If the values do not meet this requirement, the result is considered incorrect but can be recorded as qualitative. Thus it is interpreted as positive result and displayed in the “Analysis of optical measurements” column as “+” and in the conclusion as “detected”. This can be due to errors in amplification technology, in this case another PCR run is required to make semiquantitative estimation possible.
6. For samples with negative results on three detection channels (excluding tube №3) there will be conclusion “invalid” (invalid result) in the “Result” column. In this case repeating of PCR amplification, or reextraction of DNA, or resampling is needed (performed sequentially).
7. For positive and negative control samples the results must correspond to those from the Table 7.

Table 7. The results of the test for positive and negative control samples for **BacResista GLA REAL-TIME PCR Detection Kit**

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 №2-8)	+	Positive result Results of the whole series are valid
Negative control sample					
Lg value is not specified (for all tubes, for TBM: Lg≤3.5 is acceptable)	Cp is specified (for all tubes, except tube №3 which does not contain IC)	-	Lg value is not specified (for all tubes)	-	Negative result Results of the whole series are valid

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

Analysis and interpretation of PCR results for **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit**:

1. For samples with negative results on three detection channels there will be conclusion “invalid” (invalid result) in the “Result” column. In this case repeating of PCR amplification, or reextraction of DNA, or resampling is needed (performed sequentially).
2. For positive and negative control samples the results must correspond to those from the Table 8.

Table 8. The results of the test for positive and negative control samples for **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit**

Detection channel				**Result**	**Interpretation of the result**
Fam	**Hex**	**Rox**	**Cy5**		
Positive control sample					
Cp is specified	Is not considered	-	Cp is specified	+	Positive result Results of the whole series are valid
Negative control sample					
Cp is not specified	Cp is specified	-	Cp is not specified	-	Negative result Results of the whole series are valid

The principles of interpreting results are shown in Table A.2 of Annex A.

If results for negative control sample differ from those in the Table 7 (for **BacResista GLA REAL-TIME PCR Detection Kit**) and in the Table 8 (for **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit**), the results of the whole series are considered invalid. In this case decontamination is required.

If results for positive control sample differ from those in the Table 7 (for **BacResista GLA REAL-TIME PCR Detection Kit**) and in the Table 8 (for **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit**), repeat of amplification of the whole series is required.

⚠ Negative result of the test does not eliminate the probability of resistance to glycopeptyde and beta-lactam antibiotics in bacteria associated with other mechanisms of resistance[3].

11. SPECIFICATIONS

a. The analytical specificity of the **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista GLA Van/Mec 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 bacteria resistant to glycopeptides and beta-lactam antibiotics are to be registered positive for specific product through the declared detection channels.

The samples free of DNA of bacteria resistant to glycopeptides and beta-lactam antibiotics 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.

b. Analytical **sensitivity** of the **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista Gla Van/Mec 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 **BacResista GLA REAL-TIME PCR Detection Kit** and **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit** for smears/scrapes from urogenital tract in 500 µL of transport medium:

Kits for DNA extraction/elution volume, µL				
PREP-NA / 50	**PREP-GS / 100**	**PREP-NA-PLUS / 300**	**PREP-GS-PLUS / 300**	**PREP-MP-RAPID / 300**
100 copies/sample	200 copies/sample	600copies/sample		

c. Diagnostic characteristics

Number of samples (n) - 105;

Diagnostic sensitivity (95% CI) - 100% (98.7-100%);

Diagnostic specificity (95% CI) – 100% (99.8-100%).

[3] World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. 2017. Available at: http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf. Accessed 1 February 2019.

d. Data on repeatability and reproducibility

Sample	Genes of resistance in the sample	The number of conformed results	
		Repeatability (conducting PCR in the same day by the same operator, device and kit batch)	Reproducibility (conducting PCR in different days by different operators, devices and kit batches)
№1	van A\B	3 repeats of 3	4 repeats of 4
№2	ndm	3 repeats of 3	4 repeats of 4
№3	mec A	3 repeats of 3	4 repeats of 4
№4	ctx-M-1, tem, vim	3 repeats of 3	4 repeats of 4
№5	oxa-51-like, tem oxa-40-like	3 repeats of 3	4 repeats of 4

12. TROUBLESHOOTING

Table 9. 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 kits:

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 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:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

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

142281, Russia, Moscow Region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Seller: "DNA-Technology" LLC,

117587, Russia, Moscow,

int. ter. Municipal District Chertanovo Severnoye,

Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12;

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://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 limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
	Caution	VER	Version
	Do not reuse	NON STERILE	Non-sterile
EC REP	Authorized representative in the European Community	CONTROL +	Positive control

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. **BacResista GLA REAL-TIME PCR Detection Kit**

Detection channel				Result	Interpretation
Fam	Hex	Rox	Cy5		
Samples					
Lg value is specified (for one or more tubes №1,3-8)	Is not considered	-	Lg value is specified (for one or more tubes №2-8)	+	Genes associated with antibiotic resistance are detected
Lg value is not specified (for one or more tubes №1,3-8)	Cp is specified (for the same tubes as on Fam\Cy5 channels), tube №3 – does not contain IC	-	Lg value is not specified (for one or more tubes №2-8)	-	Genes associated with antibiotic resistance are not detected*
Cp is not specified (for one or more tubes №1-8)	Cp is not specified (for the same tubes as on Fam\Cy5), tube №3 – does not contain IC	-	Cp is not specified (for one or more tubes №2-8)	invalid	Invalid result

Table A.2. **BacResista Gla Van/Mec REAL-TIME PCR Detection Kit**

Detection channel			Result	Interpretation
Fam	Hex	Cy5		
Samples				
Cp is specified	Is not considered	Cp is not specified	+	Genes van A\B associated with resistance to vancomycin, teicoplanin are detected
Cp is not specified	Is not considered	Cp is specified	+	Gene mec A associated with resistance to meticillin, oxacillin is detected
Cp is specified	Is not considered	Cp is specified	+	Genes van A\B, mec A associated with resistance to vancomycin, teicoplanin, meticillin, oxacillin are detected
Cp is not specified	Cp is specified	Cp is not specified	-	Genes associated with antibiotic resistance are not detected*
Cp is not specified	Cp is not specified	Cp is not specified	invalid	Invalid result

*Negative result of the test does not eliminate the probability of resistance to glycopeptyde and beta-lactam antibiotics in bacteria associated with other mechanisms of resistanse.