



Bacterial Drug resistance

Handbook

Real Time PCR kits for detection of genetic elements responsible
for bacterial resistance to various antibiotic drugs
(Cephalosporins, Carbapenems, Glycopeptides, Macrolides)

REF see below kits table

Σ 60
96

NAME

Bacterial Drug Resistance

INTRODUCTION

Antimicrobial resistance occurs when microorganisms such as bacteria, viruses, fungi and parasites change in ways that render the medications used to cure the infections they cause ineffective. Antimicrobial resistance has been detected in all parts of the world; it is one of the greatest challenges to global public health today, and the problem is increasing. Although antimicrobial resistance is a natural phenomenon, it is being propagated by misuse of antimicrobial medicines, inadequate or inexistent programmes for infection prevention and control (IPC), poor-quality medicines, weak laboratory capacity, inadequate surveillance and insufficient regulation of the use of antimicrobial medicines.

When a microorganism is resistant to more than one drug, it is said to be multidrug-resistant (MDR). Resistance to β -lactam antibiotics which include the penicillins (oxacillin, methicillin, dicloxacillin, nafcillin etc.) and the cephalosporins make difficult to treat infections with standard types of antibiotics. In addition to β -lactam/carbapenem resistance, Enterobacteriaceae often carry genes that confer high levels of resistance to many other antimicrobials, often leaving very limited therapeutic options.

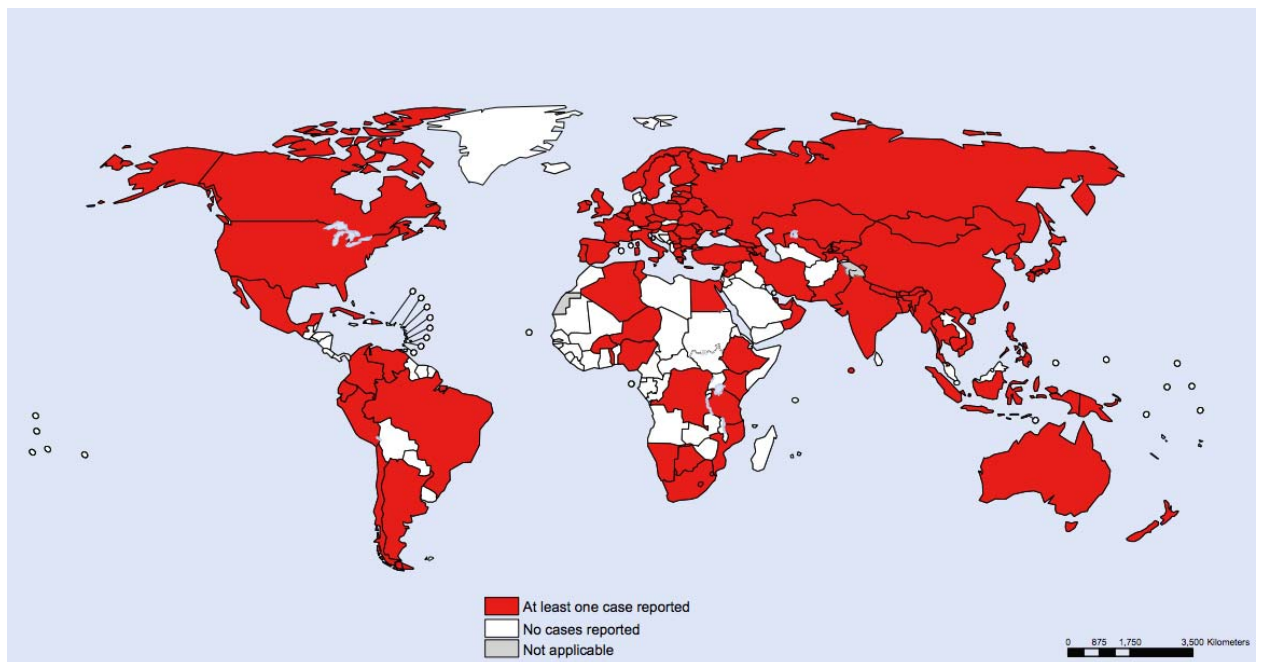


Fig.1 Drug resistance World map

INTENDED USE

Bacterial Drug Resistance Kits are intended for detection of genetic elements responsible for bacterial resistance to various antibiotic drugs (Cephalosporins, Carbapenems, Glycopeptides, Macrolides). See below table for details.

PRINCIPLE OF ASSAY

Bacterial Drug Resistance Kits are qualitative tests that allow the detection by Real Time PCR based on the amplification of the genome specific region using specific primers. In Real Time PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product. The real-time monitoring of the fluorescence intensities during the reaction allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run.

MATERIALS PROVIDED

Ready to use 0,2 ml tube format

- **60/96* ready to use 0,2 ml PCR tubes** (each PCR tube contains 21 µl of PCR)
- **Taq polymerase**, 0,5 (0,8)** ml (1 vial)
- **Negative control C-**, 0,2 ml (1 vial)
- **K+**, 0,11 ml (1 vial**)

Contains reagents for 60/96 tests.

** for 60 tests format, the product code format in below table is TXXXXX-60-T. For 96 tests format, the product code format in below table is TXXXXX-96-T*

*** 0,5 ml of Taq polymerase is present in 60 tests format; 0,8 ml of Taq polymerase is present in 96 tests format*

**** 2 vials for kit Resistance glycopeptides (Vancomycin, Teicoplanin) Enterococcus faecalis and E. Faecium (code T01787): K+ Van A, K+ Van B*

KITS TABLE

Product Code	Gene detected	Kit name and bacteria involved	Fluorescence Channels
T01781-60-T T01781-96-T	CTX-M	Resistance cephalosporins-1 <i>Enterobacteriaceae</i>	FAM: resistance gene
			HEX: Internal Control
T01782-60-T T01782-96-T	MecA	Resistance cephalosporins-2 <i>Staphylococcus aureus</i>	FAM: resistance gene
			HEX: Internal Control
T01784-60-T T01784-96-T	VIM	Resistance carbapenems-1 <i>Enterobacteriaceae</i> <i>Pseudomonas</i>	FAM: resistance gene
			HEX: Internal Control
T01785-60-T T01785-96-T	NDM	Resistance carbapenems-2 <i>Enterobacteriaceae</i>	FAM: resistance gene
			HEX: Internal Control
T01786-60-T T01786-96-T	OXA-48	Resistance carbapenems-3 <i>Enterobacteriaceae</i>	FAM: resistance gene
			HEX: Internal Control
T01746-60-T T01746-96-T	KPC	Resistance carbapenems-4 <i>Enterobacteriaceae</i>	FAM: resistance gene
			HEX: Internal Control
T01787-60-T T01787-96-T	VanA VanB	Resistance glycopeptides (Vancomycin, Teicoplanin) <i>Enterococcus faecalis</i> and <i>E. faecium</i>	FAM: VanA
			HEX: Internal Control
			ROX: VanB
T01747-60-T T01747-96-T	ErmB	Resistance macrolides-1 (macrolides, lincosamides, streptogramins) <i>Streptococcus spp,</i> <i>Staphylococcus spp</i>	FAM: resistance gene
			HEX: Internal Control
T01748-60-T T01748-96-T	Mef	Resistance macrolides-2 <i>Streptococcus spp</i>	FAM: resistance gene
			HEX: Internal Control
T01968-60-T T01968-96-T	blaDHA	Resistance cephalosporins-2	FAM: resistance gene
			HEX: Internal Control
T01960-60-T T01960-96-T	blaGES	Resistance carbapenems-5 <i>Enterobacteriaceae</i>	FAM: resistance gene
			HEX: Internal Control
T01961-60-T T01961-96-T	blaOXA23	Resistance carbapenems-6	FAM: resistance gene
			HEX: Internal Control
T01962-60-T T01962-96-T	blaOXA40	Resistance carbapenems-7	FAM: resistance gene
			HEX: Internal Control
T01965-60-T T01965-96-T	blaOXA10	Resistance cephalosporins-3	FAM: resistance gene
			HEX: Internal Control

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation

- DNA extraction kit
- Biological cabinet
- Desktop microcentrifuge for “eppendorf” type tubes
- Dry heat block
- Vortex mixer
- Pipettes
- Sterile pipette tips with filters
- 1,5 ml polypropylene sterile tubes
- Biohazard waste container
- Refrigerator, Freezer

Zone 2: Real Time amplification

- Real Time Thermal cycler
- Workstation
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 1,5/2,0 ml tubes
- Freezer, refrigerator
- Tube racks

STORAGE INSTRUCTIONS

Bacterial Drug Resistance Kits must be stored at 2-8°C. The kits can be shipped at 2-8°C and stored as indicated immediately on receipt.

STABILITY

Bacterial Drug Resistance Kits are stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

WARNINGS AND PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Use disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local authorities' regulations.
- Specimens should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

PRODUCT USE LIMITATIONS

Use of this product should be limited to personnel trained in the techniques of DNA amplification (UNI EN ISO 18113-2:2012). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Bacterial Drug Resistance Kits can analyze DNA extracted from:

- *vaginal, cervical, urethral, oropharyngeal swab*;
- *whole blood* collected in either ACD or EDTA tubes;
- *liquor* stored in "Eppendorf" tube;
- *sinovial liquid* stored in "Eppendorf" tube;
- *peritoneal and pleuric versament* stored in "Eppendorf" tube;
- *urine (sediment)*;
- *prostatic liquid* stored in "Eppendorf" tube;
- *seminal liquid*: transfer about 30 µl of seminal liquid to a polypropylene tube (1,5 ml) and add 70 µl of sterile saline solution;
- *sputum*;
- *BAL fluid*;
- *Purulent and wound discharge*;
- *Bacterial culture*

Specimens can be stored at +2-8°C for no longer than 24 hours, or freeze at -20°C to -80°C.

Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

DNA ISOLATION

The following isolation kits are recommended:

⇒ **DNA-Sorb-B** (Sacace, REF K-1-1/A) for buccal swab;

⇒ **SaMag Bacterial DNA Extraction kit** (Sacace, REF SM006);

Please carry out DNA extraction according to the manufacturer's instruction.

PROTOCOL

Bacterial Drug Resistance Kits do not include reagents required for sample preparation and DNA extraction. Blood samples and biological materials must be processed by using the recommended kits or those with similar performances of quality and quantity of extracted DNA. Use of blood samples collected in tubes containing heparin is not recommended.

The analysis of the DNA specimens using **Bacterial Drug Resistance Kits** includes the following stages:

1. Preparing the Real Time PCR;
2. Real Time PCR analysis;
3. Data analysis with the software of Real Time PCR instrument;
4. Results analysis and conclusions.

EXPERIMENTAL PROTOCOL

Total reaction volume: 35 µl

1. Prepare the necessary number of ready-to-use PCR tubes (samples + pos control + neg control).
2. Spin for 3-5 sec the **Taq polymerase**, mix by pipetting and **add 7 µl** to each PCR tube.
3. Add into the corresponding PCR tubes **7 µl** of extracted DNA from sample or control:
 - **DNA sample**Add into the corresponding PCR tubes **7 µl** of controls:
 - **C+**
 - **Negative Control C-**
4. Spin the tubes for 3–5 seconds to collect the drops.
5. Insert the tubes in the Real-time PCR instrument.

Amplification

Create a temperature profile on your instrument¹ as follows:

Step	Temperature, °C	Time	Cycles
Hold	80	2 min	1
Hold	95	1 min 30 s	1
Cycling	95	15 s	40
	60	30 s fluorescence detection	
	72	40 s	

¹ SaCycler-96™ (Sacace); Rotor-Gene™ 6000/Q (Corbett Research, Qiagen), CFX-96 / iQ5™ (BioRad); Mx3005P™ (Agilent); ABI® 7500 Real Time PCR (Applied)*;

Fluorescence is detected in FAM/Green, JOE/Yellow/HEX (and Orange/ROX) fluorescence channels.**

* To perform the test with ABI 7500 (Applied) a disposable adapter provided with the kit has to be used. Additional adapters can be purchased separately.

** select ROX channel only in kit code T01787 Resistance glycopeptides (Vancomycin, Teicoplanin) that detects VanA on FAM channel and VanB on ROX channel.

DATA ANALYSIS

The fluorescent signal intensity is detected in 2 channels (3 channels only for T01787 kit) as shown in the table below:

FAM	HEX	ROX *
Genetic element responsible for drug resistance (Positive if Ct < 35)	Internal Control Signal (Valid if Ct < 35)	VanB * (Positive if Ct<35)

* select ROX channel only in kit code T01787 Resistance glycopeptides (Vancomycin, Teicoplanin) that detects VanA on FAM channel and VanB on ROX channel.

The threshold line should cross only sigmoid curves of signal accumulation of positive samples and should not cross the baseline; otherwise, the threshold level should be raised. Set the threshold at a level where fluorescence curves are linear and do not cross curves of the negative samples.

Analysis settings for SaCycler-96 (Sacace Biotechnologies)

	Channel	Threshold	Boundary Ct Value
Internal control	HEX	10	35
All Resistance kits	FAM	100	35
Glycopeptide Resistance T01787-50(96)-T	FAM (VanA)	100	35
	ROX (VanB)	10	35

If HEX Ct value is > 35 (or absent) while FAM (or ROX) Ct value is positive the result is considered valid.

If HEX Ct value is > 35 (or absent) and FAM (or ROX) Ct value is > 35 (or absent) result is considered invalid and the sample must be tested again.

Analysis settings for RotoGene 6000/Q (Corbett Research / Qiagen)

Recommended advanced analysis settings for RotorGene 6000/Q (Corbett Research / Qiagen) can be found in the following table:

	Channel	RotorGene Analysis Settings			
		Slope correct	Outlier removal / NTC threshold	Ignore First	Threshold
Internal Control (for all kits)	Yellow	On	15%	5	0.03
Resistance (for all kits except the following)	Green	On	5%	5	0.03
Resistance glycopeptides (VanA, VanB)	Green (VanA)	On	5%	5	0.03
	Orange (VanB)	Off	10%	5	0.03
Resistance macrolides-1 (ErmB) Resistance macrolides-2 (Mef) Resistance carbapenems-3 (OXA-48)	Green	On	10%	5	0.03

If Yellow Ct value is > 35 (or absent) while FAM (or Orange) Ct value is positive the result is considered valid.

If Yellow Ct value is > 35 (or absent) and FAM (or Orange) Ct value is > 35 (or absent) result is considered invalid and the sample must be tested again.

Analysis settings for CFX-96, iQ5 (Bio-rad)










Recommended advanced analysis settings for CFX-96, iQ5 (Bio-rad) can be found in the following table:

	Channel	Threshold	Boundary Ct value
Internal control	HEX	30	35
Resistance / All kits	FAM	60	35
Macrolides Resistance -2	FAM	100	35
Glycopeptide Resistance	FAM (VanA)	60	35
	ROX (VanB)	30	35

If HEX Ct value is > 35 (or absent) while FAM (or ROX) Ct value is positive the result is considered valid.

If HEX Ct value is > 35 (or absent) and FAM (or ROX) Ct value is > 35 (or absent) result is considered invalid and the sample must be tested again.

KEY TO SYMBOLS USED

	List Number		Caution!
	Lot Number		Contains sufficient for <n> tests
	Expiration Date		Version
	Store at	NCA	Negative Control of Amplification
	Manufacturer	NCE	Negative control of Extraction
	Consult instructions for use	IC	Internal Control

* SaCycler™ is a registered trademark of Sacace Biotechnologies

* CFX-96™ is a registered trademark of Bio-Rad Laboratories

* MX3005P® is a registered trademark of Agilent Technologies

* ABI® is a registered trademark of Applied Biosystems

* Rotor-Gene™ Technology is a registered trademark of Qiagen



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