Bacterial Drug Resistance Plus

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**

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.

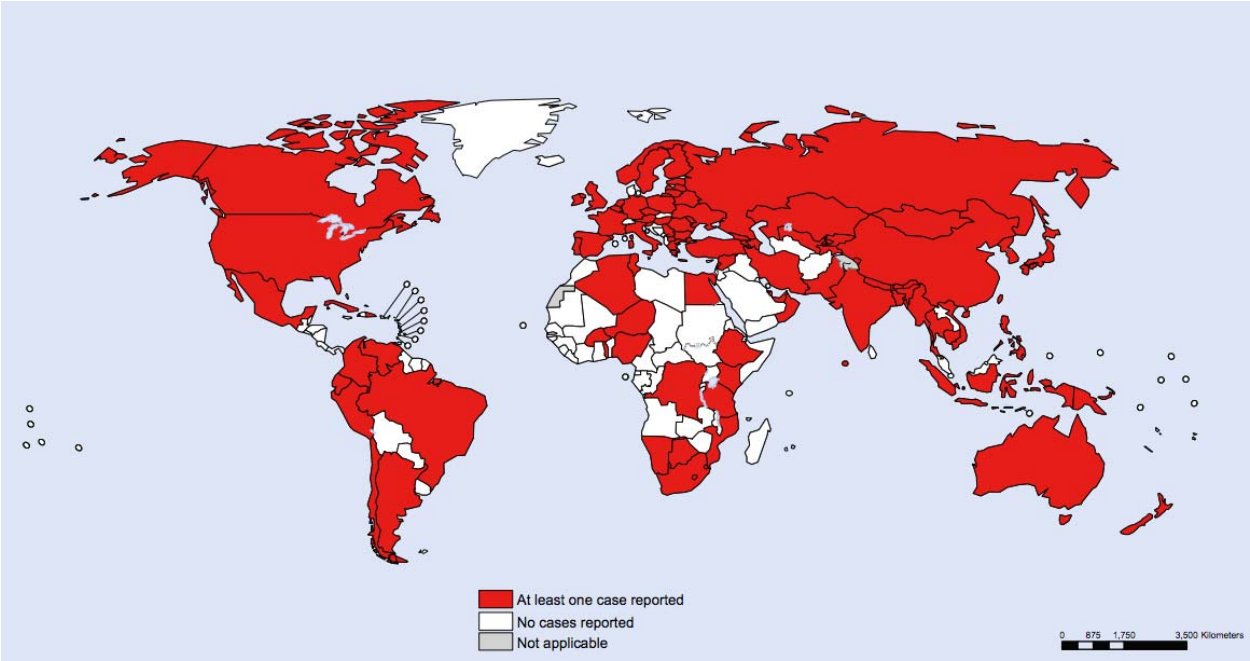

Fig.1 Drug resistance World map

INTENDED USE

Bacterial Drug Resistance Plus 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 Plus 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

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

Contains reagents for 96 tests.

KITS TABLE

Code	Gene detected	Kit name and bacteria involved	Fluorescence Channels
T01975-96-T	*Acinetobacter baumanni, blaOXA23, blaOXA40*	**Resistance CRAB** *Acinetobacter*	FAM: *Acinetobacter baumanni* HEX: *blaOXA23* ROX: *blaOXA40* Cy5: *Internal Control*
T01976-96-T	*Pseudomonas aeruginosa, blaNDM, blaVim*	**Resistance MDR** *Pseudomonas*	FAM: *Pseudomonas aeruginosa* HEX: *blaNDM* ROX: *blaVim* Cy5: *Internal Control*
T01977-96-T	*Klebsiella pneumoniae, blaOXA48, blaKPC*	***Resistance CRE Klebsiella***	FAM: *Klebsiella pneumoniae* HEX: *blaOXA48* ROX: *blaKPC* Cy5: *Internal Control*
T01978-96-T	*blaCTX-M, blaOXA10, E.coli*	***Resistance ESBL E.coli***	FAM: *blaCTX-M* HEX: *blaOXA10* ROX: *E.coli* Cy5: *Internal Control*
T01980-96-T	*Streptococcus species. Mef, ErmB*	***Resistance MLSB Streptococcus***	FAM: *Streptococcus species* HEX: *Mef* ROX: *ErmB* Cy5: *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 Plus 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 Plus 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 Plus 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: t*ransfer 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 Plus 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 Plus 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:
   - **PC+**
   - **Negative Control NC-**
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 instrument[1] 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/HEX/Yellow, ROX/ Orange and Cy5/Red 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.*

DATA ANALYSIS

After completing the amplification program, apply the following settings:

Amplifier	Parameter	Fluorescence channels and targets			
		FAM (Green)	HEX	ROX	Cy5 (Red)
CFX96 (Bio Rad)	*Analyze Data from Cycle 5 to 40 Baseline Setting – Apply Fluorescence Drift Correction*				
	Baseline threshold	60	30	60	30
	Finishing cycle (FC)	37	38	37	35
IQ5	*Base line Cycles*	Auto	5-15	5-15	Auto
	Baseline threshold	60	60	40	50
	Finishing cycle (FC)	32	33	31	31
SaCycler	*Baseline threshold*	100	10	30	50
	Finishing cycle (FC)	36	36	38	35
Rotor Gene Q (Qiagen)	*Dynamic Tube*	ON	ON	ON	ON
	Slope correct	ON	ON	ON	ON
	Ignore first	5	5	5	5
	Baseline threshold	0,02	0,02	0,02	0,02
	Outlier removal	15%	15%	15%	15%
	Eliminate cycles before	10	10	10	10
	Finishing cycle (FC)	35	35	35	35

RESULTS INTERPRETATION:

T01975-96-T, Resistance CRAB *Acinetobacter*

	FAM (Green) *Acinetobacter baumanni*	HEX (Yellow) *blaOXA23*	ROX (Orange/ Red610) *blaOXA40*	Ct Cy5 (Red) *Internal control*	RESULTS
PC	< FC	< FC	< FC	< FC	The specific reaction is passed
	NA or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	The specific reaction is not passed. Repeating of the analysis is **REQUIRED**.
NC-PCR	**NA** or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	No specific contamination.
	< FC	any	any	**NA** or > FC	Specific DNA contamination by *Acinetobacter baumannii*. Repeating of the analysis is **REQUIRED**.
	NA or > FC	< FC	< FC	**NA** or > FC	Specific DNA contamination by bla*OXA23\OXA40 genes.* Repeating of the analysis is **REQUIRED**.
	NA or > FC	**NA** or > FC	**NA** or > FC	< FC	Specific contamination by *human DNA.* Repeating of the analysis is **REQUIRED**.
Analyzed sample	< FC	**NA** or > FC	**NA** or > FC	any	**PRESENCE** of *Acinetobacter baumannii* DNA **ABSENCE** of *blaOXA23* genes DNA **ABSENCE** of *blaOXA40* genes DNA
	< FC	< FC		any	**PRESENCE** of *Acinetobacter baumannii* DNA **PRESENCE** of corresponding genes DNA.
	NA or > FC	< FC		any	**ABSENCE** of *Acinetobacter baumannii* DNA **PRESENCE** of corresponding genes DNA.
	NA or > FC	**NA** or > FC	**NA** or > FC	< FC	**ABSENCE** of *Acinetobacter baumannii* DNA **ABSENCE** of *blaOXA23* genes DNA **ABSENCE** of *blaOXA40* genes DNA
	NA or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	**Inhibition or insufficient isolation of DNA. Repeating of the analysis of this sample** REQUIRED from the extraction stage. In the case of working with samples in which there is not enough human DNA, it is recommended to use exogenous internal control (EIC) when isolated DNA from the analyzed sample.

T01976-96-T, Resistance MDR Pseudomonas

	FAM (Green) *Pseudomonas aeruginosa*	HEX (Yellow) *blaNDM*	ROX (Orange/ Red610) *blaVim*	Ct Cy5 (Red) *Internal control*	RESULTS
PC	< FC	< FC	< FC	< FC	The specific reaction is passed
	NA or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	The specific reaction is not passed. Repeating of the analysis is **REQUIRED**.
NC-PCR	**NA** or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	No specific contamination.
	< FC	any	any	**NA** or > FC	Specific DNA contamination by *Pseudomonas aeruginosa*. Repeating of the analysis is **REQUIRED**.
	NA or > FC	< FC	< FC	**NA** or > FC	Specific DNA contamination by bla*NDM\blaVim genes.* Repeating of the analysis is **REQUIRED**.
	NA or > FC	**NA** or > FC	**NA** or > FC	< FC	Specific contamination by *human DNA.* Repeating of the analysis is **REQUIRED**.
Analyzed sample	< FC	**NA** or > FC	**NA** or > FC	any	**PRESENCE** of *Pseudomonas aeruginosa* DNA **ABSENCE** of *blaNDM* genes DNA **ABSENCE** of *blaVim* genes DNA
	< FC	< FC		any	**PRESENCE** of *Pseudomonas aeruginosa* DNA **PRESENCE** of corresponding genes DNA.
	NA or > FC	< FC		any	**ABSENCE** *of Pseudomonas aeruginosa DNA* **PRESENCE** *of corresponding genes DNA.*
	NA or > FC	**NA** or > FC	**NA** or > FC	< КЦ	**ABSENCE** of *Pseudomonas aeruginosa* DNA **ABSENCE** of *blaNDM* genes DNA **ABSENCE** of *blaVim* genes DNA
	NA or > FC	**NA** or > FC	**NA** or > FC	**НЗ** или > КЦ	**Inhibition or insufficient isolation of DNA. Repeating of the analysis of this sample** REQUIRED from the extraction stage. In the case of working with samples in which there is not enough human DNA, it is recommended to use exogenous internal control (EIC) when isolated DNA from the analyzed sample.

T01977-96-T, Resistance CRE Klebsiella

	Ct FAM (Green) *Klebsiella pneumoniae*	Ct HEX (Yellow) *blaOXA48*	Ct ROX (Orange/ Red610) *blaKPC*	Ct Cy5 (Red) *Internal control*	RESULTS
PC	< FC	< FC	< FC	< FC	The specific reaction is passed
	NA or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	The specific reaction is not passed. Repeating of the analysis is **REQUIRED**.
NC-PCR	**NA** or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	No specific contamination.
	< FC	any	any	**NA** or > FC	Specific DNA contamination by *Klebsiella pneumoniae*. Repeating of the analysis is **REQUIRED**.
	NA or > FC	< FC	< FC	**NA** or > FC	Specific DNA contamination by bla*OXA48\KPC genes.* Repeating of the analysis is **REQUIRED**.
	NA or > FC	**NA** or > FC	**NA** or > FC	< FC	Specific contamination by *human DNA.* Repeating of the analysis is **REQUIRED**.
Analyzed sample	< FC	**NA** or > FC	**NA** or > FC	any	**PRESENCE** of *Klebsiella pneumoniae* DNA **ABSENCE** of *blaOXA48* genes DNA **ABSENCE** of *blaKPC* genes DNA
	< FC	< FC		any	**PRESENCE** of *Klebsiella pneumoniae* DNA **PRESENCE** of corresponding genes DNA.
	NA or > FC	< FC		any	**ABSENCE** of *Klebsiella pneumoniae* DNA **PRESENCE** of corresponding genes DNA.
	NA or > FC	**NA** or > FC	**NA** or > FC	< FC	**ABSENCE** of *Klebsiella pneumoniae* DNA **ABSENCE** of *blaOXA48* genes DNA **ABSENCE** of *blaKPC* genes DNA
	NA or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	**Inhibition or insufficient isolation of DNA. Repeating of the analysis of this sample** REQUIRED from the extraction stage. In the case of working with samples in which there is not enough human DNA, it is recommended to use exogenous internal control (EIC) when isolated DNA from the analyzed sample.

T01978-96-T, Resistance ESBL E.coli

	Ct FAM (Green) *blaCTX-M*	Ct HEX (Yellow) *blaOXA10*	Ct ROX (Orange/ Red610) *E.coli*	Ct Cy5 (Red) *Internal control*	Result
PC	< FC	< FC	< FC	< FC	The specific reaction is passed
	NA or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	The specific reaction is not passed. Repeating of the analysis is **REQUIRED**.
NC-PCR	**NA** or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	No specific contamination.
	< FC	< FC	any	**NA** or > FC	Specific DNA contamination by *blaCTX-M*\ bla*OXA10 genes.* Repeating of the analysis is **REQUIRED**.
	NA or > FC	**NA** or > FC	< FC	**NA** or > FC	Specific DNA contamination by *Escherichia coli.* Repeating of the analysis is **REQUIRED**.
	NA or > FC	**NA** or > FC	**NA** or > FC	< FC	Specific contamination by *human DNA.* Repeating of the analysis is **REQUIRED**.
Analyzed sample	**NA** or > FC	**NA** or > FC	< FC	any	**PRESENCE** of *Escherichia coli* DNA. **ABSENCE** of *blaOXA10* genes DNA. **ABSENCE** of *blaCTX-M* genes DNA.
	< FC		< FC	any	**PRESENCE** of *Escherichia coli* DNA. **PRESENCE** of corresponding genes DNA.
	< FC		**NA** or > FC	any	**ABSENCE** of *Escherichia coli* DNA. **PRESENCE** of corresponding genes DNA.
	NA or > FC	**NA** or > FC	**NA** or > FC	< FC	**ABSENCE** of *Escherichia coli* DNA. **ABSENCE** of *blaOXA10* genes DNA. **ABSENCE** of *blaCTX-M* genes DNA.
	NA or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	**Inhibition or insufficient isolation of DNA. Repeating of the analysis of this sample** REQUIRED from the extraction stage. In the case of working with samples in which there is not enough human DNA, it is recommended to use exogenous internal control (EIC) when isolated DNA from the analyzed sample.

T01980-96-T, Resistance MLSB Streptococcus

	FAM (Green) *Streptococcus species*	HEX (Yellow) *Mef*	ROX (Orange/ Red610) *ErmB*	Ct Cy5 (Red) *Internal control*	RESULTS
PC	< FC	< FC	< FC	< FC	The specific reaction is passed
	NA or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	The specific reaction is not passed. Repeating of the analysis is **REQUIRED**.
NC-PCR	**NA** or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	No specific contamination.
	< FC	any	any	**NA** or > FC	Specific DNA contamination by *Streptococcus species.* Repeating of the analysis is **REQUIRED**.
	NA or > FC	< FC	< FC	**NA** or > FC	Specific DNA contamination by *Mef\ErmB genes.* Repeating of the analysis is **REQUIRED**.
	NA or > FC	**NA** or > FC	**NA** or > FC	< FC	Specific contamination by *human DNA.* Repeating of the analysis is **REQUIRED**.
Analyzed sample	< FC	**NA** or > FC	**NA** or > FC	any	**PRESENCE** of *Streptococcus species* DNA **ABSENCE** of *Mef* genes DNA **ABSENCE** of *ErmB* genes DNA
	< FC	< FC		any	**PRESENCE** of *Streptococcus species* DNA **PRESENCE** of corresponding genes DNA.
	NA or > FC	< FC		any	**ABSENCE** of *Streptococcus species* DNA **PRESENCE** of corresponding genes DNA.
	NA or > FC	**NA** or > FC	**NA** or > FC	< FC	**ABSENCE** of *Streptococcus species* DNA **ABSENCE** of *Mef* genes DNA **ABSENCE** of *ErmB* genes DNA
	NA or > FC	**NA** or > FC	**NA** or > FC	**NA** or > FC	**Inhibition or insufficient isolation of DNA. Repeating of the analysis of this sample** REQUIRED from the extraction stage. In the case of working with samples in which there is not enough human DNA, it is recommended to use exogenous internal control (EIC) when isolated DNA from the analyzed sample.

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
	Expiration Date	VER	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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