IVD For *in Vitro* Diagnostic Use

CE

Bac Multi-Screen Real-TM

Handbook

Real Time PCR kit for detection of DNA of opportunistic bacteria of the classes Bacilli, Betaproteobacteria and Gammaproteobacteria causing nosocomial and community-acquired infections

REF R1-P028-12-S

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NAME

Bac Multi-Screen Real-TM

INTRODUCTION

Infectious diseases in the modern world remain one of the leading problems of medicine, largely determining both people's health and life expectancy. The pathogenicity of pathogens of infectious diseases is a hallmark that allows you to subdivide microorganisms into groups. Conditionally pathogenic microorganisms (UPM) include pathogens that cause the development of the infectious process only under certain conditions, and a decrease in immunity.

Some of these bacteria are represented by free-living microorganisms. They sometimes get on the epithelial surfaces or in the internal environment of a person and can cause infectious diseases (for example, Pseudomonas spp., Acinetobacter spp.).

Another group is adapted to exist in non-sterile parts of the human body associated with the environment (skin, gastrointestinal tract, upper respiratory tract). Its representatives make up the normal (endogenous) microflora of a person, but differ in virulence.

Some of them almost never cause human diseases, others often, for example, Escherichia coli, Klebsiella pneumoniae, Haemophilus influenzae.

Different types of bacteria exhibit tropism to certain anatomical niches. In this regard, each biotope of an organism has its own relatively constant flora. Being one of the protective mechanisms of the body, it can also be a reserve of pathogens of exogenous and endogenous infections. In each biocenosis, both resident and transient microorganisms are recorded, among which conditionally pathogenic representatives are of no small importance. Certain types of opportunistic bacteria are involved in the formation of the normal microflora of the microorganism and in the pathogenesis of a number of diseases.

Representatives of opportunistic bacteria can cause infectious processes under a wide variety of external and internal changes and conditions:

• taking various medications (immunosuppressants, hormones, antibacterial drugs, etc.);
• exposure to adverse environmental factors;
• decrease in immunity;
• hospital treatment;
• pregnancy and childbirth;
• advanced and children's age;
• traumatic conditions (mechanical damage to the integrity of the skin, burns, etc.);
• chronic diseases (diabetes, oncology, etc.).

Of particular interest are diseases that are caused by opportunistic microorganisms in hospitals - nosocomial (hospital) infections. The problem of such infections is currently facing healthcare, both in our country and abroad. Often, nosocomial diseases are characterized by a severe course with an increase in the length of hospital stay.

Infectious diseases that have arisen outside of a medical institution (community-acquired) are also often caused by UPM, for example, lesions of the upper and lower respiratory and urinary tracts, skin and soft tissues, etc.

The trends of recent decades include the acquisition of new properties of UPM, which lead to an increase in the severity of the disease. These primarily include the acquisition of multiple resistance to antibacterial drugs and other pathogenic features (adhesive properties and the ability to film, persistence factors).

There are traditional phenotypic and molecular genetic methods for identifying microorganisms. Phenotypic methods, establishing the ability of microorganisms to use various nutrient media as a substrate, are quite long and laborious to conduct.

For many infections caused by viruses and bacteria, molecular biological methods of diagnostics have been developed, for example, polymerase chain reaction (PCR).

Molecular genetic research allows you to identify the DNA of microorganisms, identifying them up to a given taxonomic order. Its advantage is high sensitivity, speed of obtaining results, standardization and manufacturability of research. It is important that manipulations with live bacterial cultures are not required, which serves to prevent the spread and circulation of microorganisms inside medical diagnostic and laboratory facilities.

The decision on the etiological significance of the detected UPM should not be determined by species or tribal affiliation, but on the basis of a comprehensive study in dynamics and when compared with clinical and epidemiological data.

Using the kit, the DNA of 25 bacterial representatives, often causing the development of nosocomial and community-acquired diseases, is detected. All of these microorganisms belong to 3 classes: Bacilli, Betaproteobacteria and Gammaproteobacteria.

INTENDED USE

Bac Multi-Screen Real-TM PCR kit is intended to detect DNA of different bacteria connected to opportunistic infections caused by *Streptococcus pyogenes, Citrobacter freundii, Citrobacter koseri, Burkholderia spp., Streptococcus pneumoniae, Streptococcus spp., Staphylococcus aureus, Staphylococcus spp., Klebsiella oxytoca, Klebsiella pneumoniae, Acinetobacter spp., Enterobacter cloacae, Serratia marcescens, Stenotrophomonas maltophilia, Haemophilus spp., Haemophilus influenzae, Morganella morganii, Enterobacteriales, Enterococcus spp., Escherichia coli, Pseudomonas aeruginosa, Streptococcus agalactiae, Proteus spp., Achromobacter ruhlandii, Achromobacter xylosoxidans* that cause nosocomial and community-acquired infections. Analyzed DNA should be obtained from human biological material (sputum, urine, swabs, scrapings from the respiratory tract, gastrointestinal and urogenital tract, feces, aspirates, exudates) and bacterial cultures. The principle of detection is based on real-time polymerase chain reaction method.

The kit is intended to be used as an aid for management of opportunistic infections. The results of this test should not be used as the sole basis for diagnosis, treatment or patient management decisions.

PRINCIPLE OF ASSAY

The detection of isolated DNA is performed by real-time PCR amplification of DNA of 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 which bind specifically to the amplified product. The real-time monitoring of fluorescence intensities during the real-time PCR allows detection of the amplified product without re-opening the reaction tubes after the PCR run.

The reagent kit includes mixtures for amplification, specific for the detection of DNA of opportunistic bacteria and for the determination of auxiliary analytes: DNA of all bacteria (TBM - total bacterial mass), human DNA (HMC – human material control), and also the internal control (IC), which is an indicator of the quality of the reaction in each individual tube (except tube No. 16, see table below:

To control the location of the strips in the thermalblock of the PCR device, an oligonucleotide with a fluorescent label Rox was added to the mixture for amplification of tube No. 1 and tube No. 10. It is used by the device as a marker for determining the position of strips in the thermalblock.

MATERIALS PROVIDED

Reagent	*Amount*	*Volume*
PCR-Reaction mix-1 (paraffin sealed)	12 strips x 8 tubes each	20 µl each tube
PCR-Reaction mix-2 (paraffin sealed)	12 strips x 8 tubes each	20 µl each tube
Taq Polymerase	4 tubes	500 µl each tube
Positive control (C+)	1 tube	320 µl
Cap strips	24 strips x 8 caps each	/
Negative control (C-)*	1 tube	1000 µl

Contains reagents for 12 tests

* *must be used in the isolation procedure as Negative Control of Extraction (NCE).*

MATERIALS REQUIRED BUT NOT PROVIDED

Zone 1: sample preparation:

- Biological cabinet
- Desktop microcentrifuge for “eppendorf” type tubes
- 65°C ± 2°C dry heat block
- Vortex mixer
- Pipettes with sterile, RNase-free filters tips
- 1,5 ml polypropylene sterile tubes
- Disposable gloves, powderless
- Tube racks

Zone 2: Real Time amplification:

- Real Time Thermalcycler with 4 fluorescence channels
- Workstation
- Pipettes with sterile, RNase-free filters tips
- Tube racks

STORAGE INSTRUCTIONS

Bac Multi-Screen Real-TM must be stored from +2 to +8 °C. The kit can be shipped at +2 to +8 °C for 3-4 days and should be stored at +2 to +8 °C immediately on receipt.

STABILITY

Bac Multi-Screen Real-TM is 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.

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

- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
- Use routine laboratory precautions. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas. Do not pipette by mouth.
- Do not use a kit after its expiration date.
- Do not mix reagents from different kits.
- Dispose all specimens and unused reagents in accordance with local regulations.
- The use of heparinized specimens is not recommended.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes.
- Prepare quickly the Reaction mix.
- Specimens may be infectious. Use Universal Precautions when performing the assay.
- Specimens and controls should be prepared in a laminar flow hood.
- Handle all materials containing specimens or controls according to Good Laboratory Practices in order to prevent cross-contamination of specimens or controls.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant. Follow by wiping down the surface with 70% ethanol.
- Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions 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 amplification.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification Area. Do not return samples, equipment and reagents in the area where you performed previous step. Personnel should be using proper anti-contamination safeguards when moving between areas.

PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. 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

Note: Handle all specimens as if they are potentially infectious agents.

Bac Multi-Screen Real-TM kit is intended for analysis of DNA extracted with suitable DNA purification kits from the clinical/biological materials like:

- *sputum:* take the material in an amount of at least 1.0 ml in a disposable graduated sterile vial with a wide neck and screw caps.
- *urine:* for analysis take the first portion of morning urine in an amount of not less than 20-30 ml. Urine collection is carried out in a special dry sterile container equipped with a screwed lid.
- *swabs*;
- *scrapings from the respiratory tract, gastrointestinal and urogenital tract:* sampling is carried out using special sterile disposable instruments like probes, cytobrushes or swabs, depending on the source of the clinical material and according to the established procedure. After taking the clinical material, transfer it into a tube with a suitable transport medium. Rinse the probe or swab with clinical material into the transport medium thoroughly for 10–15 sec, avoiding liquid spatter. Then remove the probe or swab from the solution, pressing it against the wall of the tube, squeeze out the excess liquid, and discard. Close the tube cap tightly and label.
- *Feces:* take a sample of feces with a mass (volume) of approximately 1-3 g (1-3 ml). A sample in the amount of 1 g (approximately) with a separate tip with a filter or disposable blades is transferred to a special sterile dry bottle. After collection of feces, close the tube cap tightly and label.
- *Aspirates:* the sampling is carried out in a disposable sterile tube. After taking the material, close the tube cap tightly and label.
- *Exudates:* sampling is carried out using special sterile disposable instruments like probes, cytobrushes or swabs, depending on the source of the clinical material and according to the established procedure. After taking the clinical material, transfer it into a tube with a suitable transport medium. Rinse the probe or swab with clinical material into the transport medium thoroughly for 10–15 sec, avoiding liquid spatter. Then remove the probe or swab from the solution, pressing it against the wall of the tube, squeeze out the excess liquid, and discard. Close the tube cap tightly and label.
- *bacterial cultures:* material is taken from liquid and solid media using a disposable microbiological loop or spatula. Place a single colony of cells or 100 µl of growth medium in a 1.5-2.0 ml vial with 500 µl containing a physiological saline solution. After taking the material, close the tube cap tightly and label.

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

DNA ISOLATION

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the "SAMPLE COLLECTION, STORAGE AND TRANSPORT" paragraph, could be used.

Sacace Biotechnologies recommends to use the following kits:

- ⇨ **DNA/RNA Prep NA** (Sacace, REF K-2-9/2);
- ⇨ **SaMag Bacterial DNA Extraction kit** (Sacace, REF SM006).

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

A negative control sample should go through all stages of DNA extraction. Sterile physiological saline solution can be used as a negative control sample with the volume according to DNA extraction kit used. If amplification is not performed in the same day of extraction, the processed samples can be stored at 2-8°C for at maximum period of 5 days or frozen at –20°/-80°C.

PROTOCOL (Reaction volume 35 µl):

PREPARING TUBES FOR PCR

1. Take out from the refrigerator all the reagents.
2. For each clinical sample and controls are required 1 strip of **PCR-Reaction mix-1** and 1 strip of **PCR-Reaction mix-2**. Prepare the required number of strips, including N strips for N clinical samples, for Positive Control Amplification (PCA) and for Negative Control of Extraction (NCE).

Example: to test 2 samples, mark 8 strips - 4 strips for the samples, 2 strips for "C+" and 2 strips for "C-". See Table 3 for reference.

Table – Example of strip marking for PCR procedure

Samples	Ordinal number of strip	Type of strip
Sample A	1	Strip mix-1
	2	Strip mix-2
Sample B	3	Strip mix-1
	4	Strip mix-2
C-	5	Strip mix-1
	6	Strip mix-2
C+	7	Strip mix-1
	8	Strip mix-2

3. Vortex shortly and spin for 3-5 sec the **Taq polymerase** tube. Mix by pipetting and **add 10 µl** to each PCR tube without damaging the paraffin layer.
4. Add **5 µl** of **DNA samples** isolated from the clinical samples to each PCR tube without damaging the paraffin layer.
5. Run the **control reactions**:

C– - Add **5 µl** of the **Negative** extracted from the Negative Control sample to the tube labeled NCE (Negative Control of Extraction) without damaging the paraffin layer.

C+ - Add **5 µl** of **Positive control (C+)** to the tube labeled C+ (Positive Control of Amplification) without damaging the paraffin layer.

6. Cap and spin down briefly (2-3 seconds) all the strips.
7. Transfer the tubes into the thermalcycler. First tube of strip **PCR-Reaction mix-1** and second tube of strip of **PCR-Reaction mix-2** contain blue buffer.

AMPLIFICATION

1. Create a temperature profile on your instrument as follows:

Step	Temperature °C	Min.	Sec.	Repeats	Fluorescence signal detection**
1	80	0	30	1	
	94	1	30		
2	94	0	30	5	
	64	0	15		
3	94	0	10	45	
	64	0	15*		√

*For example, SaCycler-96™ (Sacace), CFX-96™*** Deep Well / iQ5™ (BioRad); Mx3005P™/Mx3000P™ (Agilent), ABI® 7500 Real Time PCR (Applied Biosystems);*
** On ABI® 7500 Real Time PCR instrument, please set the fluorescence acquisition time to 30 seconds.*
*** Fluorescence detection on channels FAM/Green, Joe/HEX/Yellow, ROX/Orange, Cy5/Red*
***NOTE:* FOR CFX-96** *and other plate type instruments: it is recommended to use at least two additional empty strips placing them in the last left and right columns of the thermal block to better uniform the thermolid pressure in case of not filling the complete plate.*

Targets are detected by a fluorescent signal in the channels FAM, HEX, ROX and Cy5 fluorophores according to the table below:

PCR-Reaction mix	Tube N°	Fam	Hex	Rox	Cy5	Colour mixture
«PCR-Reaction mix-1»	1	TBM*	IC	Marker	-	Blue
	2	Streptococcus pyogenes	IC	-	-	Colorless
	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	-	-	
«PCR-Reaction mix-2»	9	Enterobacter cloacae	IC	-	Serratia marcescens	Colorless
	10	Stenotrophomonas maltophilia	IC	Marker	Haemophilus spp.	Blue
	11	Haemophilus influenzae	IC	-	-	Colorless
	12	Morganella morganii	IC	-	Enterobacteriales	
	13	Enterococcus spp.	IC	-	HMC	
	14	Escherichia coli	IC	-	Pseudomonas aeruginosa	
	15	Streptococcus agalactiae	IC	-	Proteus spp.	
	16	Achromobacter ruhlandii	-	-	Achromobacter xylosoxidans	

** TBM, Total Bacterial Mass*

The **PCR-Reaction mix** strips 1 and 2 include probes and primers for amplification of the specific regions for the detection of DNA of opportunistic bacteria and for the determination of auxiliary analytes such as DNA of all bacteria (TBM -total bacterial mass), human DNA (HMC – human material control), and also the internal control (IC), which is an indicator of the quality of the reaction in each individual tube (except tube No. 16).
To control the location of the strips in the thermalblock of the PCR device, an oligonucleotide with a fluorescent Rox dye has been added to the mixture for amplification of tube No. 1 and tube No. 10. This marker is used by the device as a marker for determining the position of strips in the thermalblock.

INSTRUMENT SETTINGS

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, approximately 10% of the fluorescence value in the last amplification cycle for the positive control of amplification.

DATA ANALYSIS

The results are interpreted by the real-time PCR instrument software by the crossing or not crossing of the threshold line by the fluorescence curve (in the middle of the linear section of the fluorescence curve for the positive control (C+) in logarithmic coordinates).
The result of amplification is considered **positive** if the fluorescence curve is characteristic of real-time PCR (S-shaped) and crosses the threshold line.
The result of amplification is considered **negative** if the fluorescence curve is not S-shaped and if it does not cross the threshold line (the Ct value is absent).

Automatic results are available only on *SaCycler-96™ (Sacace)* Real-Time PCR software.

RESULTS INTERPRETATION

The reaction results are analysed automatically using the software supplied with the SaCycler-96™ Real Time PCR device.
After completion of PCR, the program displays in the table in the column "Result": "+" or "-".
According to the PCR results (threshold cycles), the software automatically calculates the concentration logarithms, which are indicated in the line with the name of the corresponding microorganism and TBM. Based on the results of automatic comparison of the values of the

logarithms, is made an assessment of the interval of the percentage of each microorganism from the total bacterial mass in the DNA preparation, which is indicated in the results column.
If the logarithm of the TBM is greater than 7, for a more accurate semi-quantitative assessment, it is recommended to dilute the DNA preparation 10-100 times and re-amplify it.
Based on the principle of automatic interpretation of results a logarithm value less than 2.5 is interpreted as negative, while in the window "Analysis of optical measurements" the result is indicated as "-", and interpreted as - "not detected".
For each reaction, is automatically calculated an efficiency coefficient factor. If the coefficient values are outside the boundary limits, the result of the reaction is interpreted as unreliable (nd). In this case, the column of results will indicate “-”. This result may be associated with low efficiency in the amplification reaction or sample preparation and in this case is suggested to repeat the PCR or perform a new DNA isolation and PCR stage, or, if needed a resampling of the clinical material.

If the value of the logarithm of the TBM is not specified, and the values of the logarithms for the detected microorganisms are indicated, then the determination of the percentage of microorganisms is impossible. This result is interpreted as unreliable (nd) and may be associated with low efficiency of amplification or sample preparation, in this case is required to repeat the PCR with an existing DNA preparation, or perform again the DNA isolation and PCR, or resampling of clinical material (performed sequentially).

The lg value indicated when identifying each microorganism shall be less than the lg TBM + 0.5 value. If this condition is not met, the determination of the percentage of microorganisms is not correct. This result is interpreted as unreliable (nd) and may be associated with low efficiency of amplification or sample preparation, in this case is required to repeat the PCR with an existing DNA preparation, or perform again the DNA isolation and PCR, or resampling of clinical material (performed sequentially).

In case of negative results obtained in one test tube simultaneously for IC and for specific indicators recorded in three detection channels (excluding test tube without IC, No. 16 Strip No. 2), the column “Result” will indicate “nd”. The result of the study for this sample cannot be correct, due to low efficiency of amplification or sample preparation, in this case is required to repeat the PCR with an existing DNA preparation, or perform again the DNA isolation and PCR, or resampling of clinical material (performed sequentially).

For quality control of the preanalytical stage and DNA extraction from samples containing human biological material, is used an additional HMC indicator (test tube No. 13, Strip No. 2). In the absence of specific positive results in all test tubes and a log HMC value of less than 2.5, a negative result should be interpreted as “insufficient material for the study.” In this case, is required to repeat the PCR with an existing DNA preparation, or perform again the DNA isolation and PCR, or resampling of clinical material (performed sequentially).

If the test material does not have to contain human DNA or the specificity of the test material could show some trace amount (for example, pure cultures of bacteria or blood culture), the HMC value is not taken into account.

Table for controls results:

Detection channels				Result	Interpretation of the result
FAM	**HEX**	**ROX**	**CY5**		
Positive control sample					
Ct value defined (for all tubes)	Ct value not defined	-	Ct value defined (for test tubes N° 3,5-7,9,10,12-16)	+	Positive result
Negative control sample					
lg for TBM not specified or ≤3.5; for others - lg not specified	Cp value defined and <35 *, test tube N° 16 - without IC).	-	lg not specified	-	"Less than 3.5 lg" for TBM or negative
* other results than the ones shown in the table are interpreted as an unreliable (nd) and may be associated with inefficiency of the amplification reaction. in which case, repeated PCR is required.					

PERFORMANCE

Sensitivity

The detection limit for bacterial DNA is 10 copies per amplification tube (2.0 x 10^3 copies / ml DNA preparation). The detection limit is established by analyzing serial dilutions of laboratory control samples (LCS)

The detection limit depends on the type of biomaterial, the kit / set of reagents used for DNA isolation and the final volume of elution (dilution) of the extracted DNA.

Diagnostic characteristics

Type of biomaterial	Number of samples	Diagnostic sensitivity	Diagnostic specificity
phlegm	12*	100% (79,41 - 100)	100% (98,71 - 100)
urine	63	100% (97,14 - 100)	100% (99,75 - 100)
swabs/scrapes from respiratory tract	18	100% 90,51 - 100)	100% (99,11 - 100)
swabs/scrapes of epithelial cells from gastrointestinal tract	9	100% 86,77 - 100)	100% (98,16 - 100)
swabs/scrapes from urogenital tract	7	100% 81,47 - 100)	100% (98,16 - 100)
faeces	6	100% (81,47- 100)	100% (97,24 - 100)
aspirates	9	100% (82,35- 100)	100% (98,23 - 100)
exudates	8	100% (80,49 - 100)	100% (98,00 - 100)
Total	**132**	**100% (98,68 - 100)**	**100% (99,88 - 100)**
bacterial cultures	**46**	**100% (95,07 – 100)**	**100% (99,66 – 100)**

Specificity

The analytical specificity of **Bac Multi-Screen Real-TM** PCR kit was confirmed in clinical studies and was checked against bacterial strains.

The analytical specificity of the **Bac Multi-Screen Real-TM** 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 the detected bacteria are to be registered positive for specific product through the declared detection channels.

The samples free of DNA of the detected bacteria 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.

There are not non-specific positive results of amplification of DNA sample in the study of high concentrations of DNA (at least $2x10^3$ copies per amplification tube) of conditionally pathogenic and other microorganisms that cause infectious diseases, and/or normally present in the loci of biomaterial sampling: S. pyogenes, C. freundii, C. koseri, Burkholderia spp., S. pneumoniae, Streptococcus spp., S. aureus Staphylococcus spp., K. pneumoniae, K. oxytoca, Acinetobacter spp., E. cloacae, S. marcescens, S. maltophilia, Haemophilus spp., H. influenzae, M. morganii, Enterococcus spp., Campylobacter spp., Candida spp., Chlamydophila pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Salmonella spp., as well as human DNA.

TROUBLESHOOTING

- The absence of positive signal in C+ in channels FAM, HEX, ROX and Cy5 may indicate incorrect amplification program or other errors made during PCR amplification. In this case, PCR should be carried out once again
- Detection of any Ct value in NCE (except in HEX channel) suggests contamination of reagents or samples. In this case, it is necessary to repeat the analysis of all tests starting from the isolation stage and to take measures for detecting and eliminating the source of contamination.

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	For *in Vitro* Diagnostic Use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**NCE**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

* SaCycler™ is a registered trademark of Sacace Biotechnologies
* CFX™ and iQ5™ are registered trademarks of Bio-Rad Laboratories
* MX3005P® is a registered trademark of Agilent Technologies
* ABI® is a registered trademark of Applied Biosystems

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