ProstateScreen Real-TM

Handbook

Real Time PCR test for diagnosis of chronic bacterial prostatitis (*Enterobacter spp., Klebsiella spp., Enterococcus faecalis, E. faecium, Escherichia coli, Proteus spp., Pseudomonas aeruginosa, Serratia spp., Staphylococcus aureus, Streptococcus spp.*)

REF 01765-50

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NAME

ProstateScreen Real-TM

INTRODUCTION

Urinary tract infections can cause disease both in upper and lower urinary system. They can lead to different pathologies, pyelonephritis, cystitis, urethritis and prostatitis which among the diseases of the prostate have a special place.

Prostatitis disease is widespread mostly in reproductive age men from 20 to 45 years. Prostatitis usually are not life threatening, but have a long and low effectiveness rate of the therapy and may cause sexual dysfunctions. Depending on the etiology of prostatitis, they are divided into infectious and congestion (congestive). Congestive prostatitis can be due to the stagnation of prostatic secretions and semen, and also to venous congestion in the pelvic organs and scrotum.

Chronic prostatitis (chronic pelvic pain syndrome) - is one of the most common, difficult to diagnose and treat conditions in urology. Numerous studies show that chronic prostatitis associated with different pathological conditions, such as benign prostatic hyperplasia and prostate cancer, can cause problem in its diagnosis and treatment.

The leading role in the occurrence of bacterial prostatitis and urogenital infections in men is played by Escherichia coli, the other members of the family Enterobacteriacea (*Klebsiella spp., Enterobacter spp., Proteus spp., Serratia spp.*), *Pseudomonas aeruginosa, Streptococcus spp.* and *Staphylococcus spp*. Sacace has developed a comprehensive diagnostic test system for the determination of the bacterial flora, under the commercial name "ProstateScreen" (tab. 1).

Table 1. Microorganisms identified test system "ProstateScreen."

Gram Negative Bacilli	*Escherichia coli*
	Enterobacter spp., Klebsiella spp.
	Proteus spp.
	Serratia spp.
	Pseudomonas aeruginosa
Gram Positive Cocci	*Enterococcus faecalis* and *E .faecium*
	Staphylococcus aureus
	Streptococcus spp.

INTENDED USE

ProstateScreen Real-TM PCR kit is an in vitro nucleic acid amplification test intended for detection of *Escherichia coli, Enterobacter spp., Klebsiella spp., Proteus spp., Serratia spp., Pseudomonas aeruginosa, Enterococcus faecalis, E. faecium, Staphylococcus aureus, Streptococcus spp*.

PRINCIPLE OF ASSAY

ProstateScreen Real-TM is qualitative test 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.

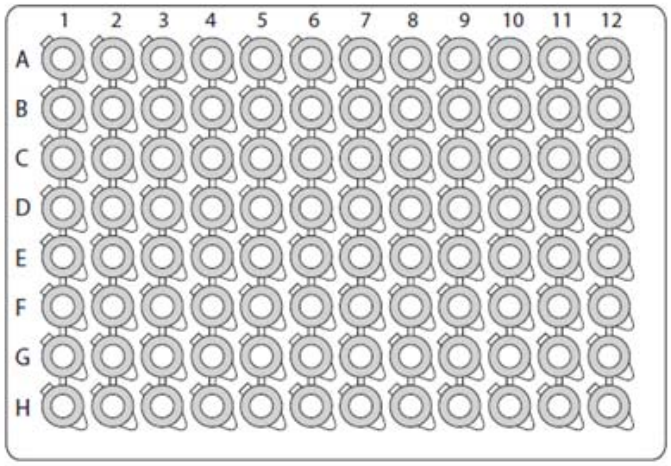
This kit uses endogenous Internal Control which is present in each reaction tube containing PCR mastermix. The endogenous IC detects human genomic DNA sequence which must always be present in each extracted sample.

This approach allows to monitor not only possible reaction inhibition but also:

- correct collection of clinical specimens;
- effectiveness of sample preparation;
- errors in the analysis (sample not added in the amplification mixture);

MATERIALS PROVIDED

- **12 x 8 strip ready to use** (each PCR tube contains 21 µl)



Amplification Mixes

⇦ A Escherichia coli
⇦ B Enterobacter spp., Klebsiella spp
⇦ C Proteus spp
⇦ D Serratia spp.
⇦ E Pseudomonas aeruginosa
⇦ F Enterococcus faecalis, E. faecium
⇦ G Staphylococcus aureus
⇦ H Streptococcus spp.

- Positive Controls Panel, 8 tubes, 110 µl;
- Neg Control, 200 µl;
- Taq-Polymerase, 1 x 800 µl.

Contains reagents for 12 tests.

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

ProstateScreen Real-TM 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

ProstateScreen Real-TM 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

ProstateScreen Real-TM Kits can analyze DNA extracted from:

- *urine sediment* : collect 10-20 ml of first-catch urine in a sterile container. Centrifuge for 30 min at 3000 x g, carefully discard the supernatant and leave about 200 µl of solution. Resuspend the sediment. Use the suspension for the DNA extraction.
- *prostatic liquid* stored in “Eppendorf” tube;
- *seminal liquid:* maintain semen for 40 min in darkness until liquefaction. Use 100 µl for the DNA extraction.

It is recommended to process samples immediately after collection. Store samples at 2–8 °C for no longer than 24 hours, or freeze at –20/80°C. 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, 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-Sorb-A** (Sacace, REF K-1-1/A);

⇒ **SaMag STD DNA Extraction kit** (Sacace, REF SM007).

Please carry out the DNA extraction according to the manufacturer’s instructions.

PROTOCOL

ProstateScreen Real-TM kit does 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 genomic DNA specimens using **ProstateScreen Real-TM** kit 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.
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:
   - **DNA** sample

   Add into the corresponding PCR tubes **7 µl** of controls:
   - **C+ Pos Control**
   - **Negative Control C-**

** it's suggested to use at least 1 Positive and 1 Neg Control for each run.*

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* as follows:

	Temp °C	Time (s)	Cycles
Hold	80	120 s	1
Hold	95	90 s	1
Cycling	95	15 s	40
	60	**30 s fluorescence detection**	
	72	40 s	

* *For example,SaCycler-96™ (Sacace), CFX-96 / iQ5™ (BioRad); Mx3005P™ (Agilent); ABI® 7500 Real Time PCR (Applied)**; RotorGene Q (Qiagen)****

Fluorescence is detected in FAM/Green (pathogen), JOE/Yellow/HEX (Internal Control) 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.*

**** NOTE: If the test is performed on Rotor Gene (Qiagen) instrument the tube caps may be marked and it's recommended to cut the strip into two equal parts (4 tubes), but it is necessary to strictly observe the order of the tubes in the rotor*

DATA ANALYSIS

The fluorescent signal intensity is detected in 2 channels as shown in the table below:

FAM / Green	HEX / Yellow
Specific pathogen signal (Positive if Ct < Boundary Value)	**Internal Control Signal (Valid if Ct < Boundary Value)**

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.

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

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

Analysis settings for CFX96™ (Bio-Rad)

	Channel	Threshold	Boundary Ct Value
Internal control	HEX	30	35
Escherichia coli	FAM	60	33
Proteus spp.	FAM	60	35
Pseudomonas aeruginosa	FAM	60	35
Enterococcus faecalis, E. faecium	FAM	60	35
Enterobacter spp., Klebsiella spp.	FAM	60	35
Serratia spp.	FAM	60	35
Staphylococcus aureus	FAM	60	35
Streptococcus spp.	FAM	60	33

Analysis settings for SaCycler-96™ (Sacace Biotechnologies)

	Channel	Threshold	Boundary Ct Value
Internal control	HEX	20	35
Escherichia coli	FAM	100	33
Proteus spp.	FAM	100	35
Pseudomonas aeruginosa	FAM	100	35
Enterococcus faecalis, E. faecium	FAM	100	35
Enterobacter spp., Klebsiella spp.	FAM	100	35
Serratia spp.	FAM	100	35
Staphylococcus aureus	FAM	100	35
Streptococcus spp.	FAM	100	33

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

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

Analysis settings (Rotor-Gene Q)

	Channel	Dynamic tube	Slope Correct	NTC Threshold	Threshold	Boundary Ct Value
Internal control	Yellow	on	on	15%	0,03	35
Escherichia coli	Green	on	on	15%	0,02	31
Proteus spp.	Green	on	on	15%	0,02	35
Pseudomonas aeruginosa	Green	on	on	15%	0,02	35
Enterococcus faecalis, E. faecium	Green	on	on	15%	0,02	35
Enterobacter spp., Klebsiella spp.	Green	on	on	15%	0,02	35
Serratia spp.	Green	on	on	15%	0,02	35
Staphylococcus aureus	Green	on	on	15-25%	0,02	35
Streptococcus spp.	Green	on	on	15-25%	0,02	31

If Yellow Ct value is > Boundary Ct value (or absent) while FAM/Green Ct value is positive the result is considered valid.

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

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	**POS**	Positive Control of Amplification
	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

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