Sacace BIOTECHNOLOGIES

CE 1023

IVD For *in Vitro* Diagnostic Use

For Professional Use Only

Chlamydia trachomatis Real-TM

Handbook

Real Time PCR kit for qualitative detection of Chlamydia trachomatis

REF B1-100FRT

Σ 100

NAME

Chlamydia trachomatis Real-TM

INTRODUCTION

STDs (sexually transmitted diseases) refer to a variety of bacterial, viral and parasitic infections that are acquired through sexual activity. Some STDs, such as syphilis and gonorrhea, have been known for centuries — while others, such as HIV, have been identified only in the past few decades. STDs are caused by more than 25 infectious organisms. As more organisms are identified, the number of STDs continues to expand. Common STDs include: chlamydia, gonorrhea, herpes, HIV, HPV, syphilis, gardnerella and trichomoniasis.
The Chlamydia trachomatis is nonmotile, gram-negative bacterial pathogen and is the most common sexually transmitted bacterial agent. The prevalence of C. trachomatis infection in sexually active adolescent women, the population considered most at risk, generally exceeds 10%, and in some adolescent and STD clinic populations of women, the prevalence can reach 40%. The prevalence of C. trachomatis infection ranges from 4 to 10% in asymptomatic men and from 15 to 20% in men attending STD clinics. Chlamydial infections in newborns occur as a result of perinatal exposure; approximately 65% of babies born from infected mothers become infected during vaginal delivery.
The development of tests based on nucleic acid amplification technology has been the most important advance in the field of STD diagnosis. Because nucleic acid amplification is exquisitely sensitive and highly specific, it offers the opportunity to use noninvasive sampling techniques to screen for infections in asymptomatic individuals who would not ordinarily seek clinical care.

INTENDED USE

Chlamydia trachomatis Real-TM PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Chlamydia trachomatis* DNA in the clinical materials (urogenital, rectal, and throat swabs; eye discharge; urine; and prostate gland secretion) by means of real-time hybridization-fluorescence detection.

The results of PCR analysis are taken into account in complex diagnostics of disease.

PRINCIPLE OF PCR DETECTION

Chlamydia trachomatis detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific primers. In real-time PCR the amplified product is detected by 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 the detection of accumulating product without re-opening the reaction tubes after the PCR run. **Chlamydia trachomatis Real-TM** PCR kit is a qualitative test that contains the Internal Control (IC), which must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition. **Chlamydia trachomatis Real-TM** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by chemically modified polymerase (TaqF), which is activated by heating at 95 ºC for 15 min.

CONTENT

Reagent	***Description***	***Volume (ml)***	***Amount***
PCR-mix-1-FL *Chl. trachomatis*	colorless clear liquid	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C-)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

**must be used in the extraction procedure as Negative Control of Extraction.*

*** add 10 µl of Internal Control-FL during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-A **REF** K-1-1/A protocol).*

ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers up to 200 µl.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Real Time PCR instrument.
- Disposable polypropylene microtubes for PCR or PCR-plate.
- Refrigerator for 2–8 ℃.
- Deep-freezer for ≤ –16 ℃.
- Waste bin for used tips.

GENERAL PRECAUTIONS

IVD

***In Vitro* Diagnostic Medical Device**
For *In Vitro* Diagnostic Use Only

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.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- 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.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

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.

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 (EN375). 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.

STORAGE INSTRUCTIONS

All components of the **Chlamydia trachomatis Real-TM** PCR kit (except for Polymerase (TaqF) and PCR-mix-2-FRT) are to be stored at 2–8 ºC when not in use. The **Chlamydia trachomatis Real-TM** kit can be shipped at 2-8℃ but should be stored at 2-8℃ and -20℃ immediately on receipt. The shelf life of reag ents before and after the first use is the same, unless otherwise stated.

Polymerase (TaqF) and PCR-mix-2-FRT should be stored at or below minus 16 ºC

PCR-mix-1-FL *Chlamydia trachomatis* should be kept away from light

STABILITY

Chlamydia trachomatis 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.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

Chlamydia trachomatis Real-TM can analyze DNA extracted from:

- *cervical, urethral, conjunctival swabs:* insert the swab into the nuclease-free 1,5 ml tube and add 0,2 ml of Transport medium. Vigorously agitate swabs for 15-20 sec.
- *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..

It is recommended to process samples immediately after collection. Store samples at 2–8 ℃ for no longer than 24 hours, or freeze at –20/80 ℃. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

DNA ISOLATION

The following isolation kit is recommended:

⇒ **DNA-Sorb-A** (Sacace, REF K-1-1/A).

Please carry out the DNA extraction according to the manufacturer's instructions. Add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture.

(Note: the Sacace Internal Control is the same for all urogenital infectious kits)

REAGENTS PREPARATION (REACTION VOLUME 25 µL):

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

1. Thaw the **PCR-mix-2-FRT** tube. Vortex the tubes with **PCR-mix-1-FL *Chamydia trachomatis*, PCR-mix-2-FRT, and polymerase (TaqF)** and then centrifuge briefly. Take the required number of strip or unstrip tubes for amplification of DNA from clinical and control samples (0.2-ml tubes for a 36-well rotor or 0.1-ml strip tubes for a 72-well rotor).
2. For N reactions (including 2 controls), add to a new tube:
   10*(N+1) µl of **PCR-mix-1-FL *Chlamydia trachomatis***,
   5.0*(N+1) µl of **PCR-mix-2-FRT,**
   0.5*(N+1) µl of **polymerase (TaqF).**
   Vortex the tube and then centrifuge briefly. Transfer **15 µl** of the prepared mixture to each tube.
3. Using tips with aerosol barrier, add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction to the prepared tubes.
4. Perform control amplification reactions:

NCA -Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ -Add **10 µl** of **Positive Control complex** (to the tube labeled C+ (Positive Control of Amplification).

C– -Add **10 µl** of a sample extracted from the **Negative Control** to the tube labeled C- (Negative Control of Extraction).

Chlamydia trachomatis is detected on the FAM (Green) channel, *IC DNA* on the JOE(Yellow)/HEX/Cy3 channel

Amplification

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

	Rotor-type Instruments[1]			Plate- or modular type Instruments[2]		
Step	***Temperature, °C***	***Time***	**Repeats**	***Temperature, °C***	***Time***	**Repeats**
1	**95**	15 min	1	**95**	15 min	1
2	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
3	**95**	5 s	40	**95**	5 s	40
	60	20 s *fluorescent signal detection*		**60**	30 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

[1] *For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)*
[2] *For example, iQ5™ (BioRad); Mx3005P™ (Stratagene), Applied Biosystems® 7300/7500/StepOne Real Time PCR (Applera), SmartCycler® (Cepheid), LineGeneK® (Bioer)*

Fluorescence is detected at the 2nd step of Cycling 2 stage (60 ℃) in FAM/Green and JOE/Yellow/Hex/Cy3 fluorescence channels.

INSTRUMENT SETTINGS

Rotor-type instruments (RotorGene 3000/6000, RotorGene Q)

Channel	Threshold	More Settings/ Outlier Removal	Slope Correct
FAM/Green	*0.1*	*0 %*	*Off*
JOE/Yellow	*0.1*	*5 %*	*Off*

Plate-type instruments (iQ5, Mx300P, ABI 7500/7300)

For result analysis, set the threshold line at a level corresponding to 10–20% of the maximum fluorescence signal obtained for Pos C+ sample during the last amplification cycle.

DATA ANALYSIS

The fluorescent signal intensity is detected in two channels:

– The signal from the *Chlamydia trachomatis* DNA amplification product is detected in the FAM/Green channel;

– The signal from the Internal Control amplification product is detected in the JOE/Yellow/HEX/Cy3 channel.

Interpretation of results

The results are interpreted by the software of the instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

Principle of interpretation:

— *Chlamydia trachomatis* DNA is **detected** in a sample if its Ct value is present in the FAM channel. The fluorescence curve should cross the threshold line in the area of exponential fluorescence growth.

— *Chlamydia trachomatis* DNA is **not detected** in a sample if its Ct value is absent in the FAM channel (fluorescence curve does not cross the threshold line) while the Ct value in the JOE channel is less than 33.

— The result is **invalid** if the Ct value of a sample in the FAM channel is absent while the Ct value in the JOE channel is either absent or greater than the specified boundary value (Ct > 33). It is necessary to repeat the PCR analysis of such samples.

The result of analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (Table 2).

Table 2. Results for controls

Control	Stage for control	Ct channel Fam	Ct channel Joe	Interpretation
NCE	DNA isolation	NEG	POS	Valid result
NCA	Amplification	NEG	NEG	Valid result
C+	Amplification	POS	POS	Valid result

QUALITY CONTROL PROCEDURE

A defined quantity of Internal Control (IC) is introduced into each sample and control at the beginning of sample preparation procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

A negative control of extraction (NCE), negative amplification control (NCA), positive amplification control (C+) are required for every run to verify that the specimen preparation, the amplification and the detection steps are performed correctly.

If the controls are out of their expected range (see table Results for Controls), all of the specimens and controls from that run must be processed beginning from the sample preparation step.

SPECIFICATIONS

Sensitivity

The analytical sensitivity of **Chlamydia trachomatis Real-TM** PCR kit is specified in the table below.

Clinical material	DNA extraction kit	Analytical sensitivity, GE/ml*
Urogenital swabs	DNA-sorb-A	5×10^2
Urine	DNA-sorb-A	1×10^3

** Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.*

Specificity

The analytical specificity of **Chlamydia trachomatis Real-TM** PCR kit is ensured by selection of specific primers and probes as well as by selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. There were no nonspecific responses during examination of human DNA as well as DNA panel of the following microorganisms: *Gardnerella vaginalis, Lactobacillus spp., Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Candida albicans, Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma genitalium, Neisseria flava, Neisseria subflava, Neisseria sicca, Neisseria mucosa, Neisseria gonorrhoeae, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV* type 1 and 2, *CMV,* and *HPV.*

TROUBLESHOOTING

1. Weak or no signal of the IC (Joe/Hex/Cy3 channel) for the Negative Control of extraction.
    - The PCR was inhibited.
        - ⇒ Make sure that you use a recommended DNA extraction method and follow to the manufacturer's instructions.
        - ⇒ Re-centrifuge all the tubes before pipetting of the extracted DNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. Don't disturb the pellet, sorbent inhibit reaction.
    - The reagents storage conditions didn't comply with the instructions.
        - ⇒ Check the storage conditions
    - Improper DNA extraction.
        - ⇒ Repeat analysis starting from the DNA extraction stage
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the PCR conditions and select for the IC detection the fluorescence channel reported in the protocol.
    - The IC was not added to the sample during the pipetting of reagents.
        - ⇒ Make attention during the DNA extraction procedure.
2. Weak or no signal of the Positive Control.
    - The PCR conditions didn't comply with the instructions.
        - ⇒ Check the amplification protocol and select the fluorescence channel reported in the manual.
3. Fam (Green) signal with Negative Control of extraction.
    - Contamination during DNA extraction procedure. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol.
        - ⇒ Use only filter tips during the extraction procedure. Change tips between tubes.
        - ⇒ Repeat the DNA extraction with the new set of reagents.
4. Any signal with Negative Control of PCR (DNA-buffer).
    - Contamination during PCR preparation procedure. All samples results are invalid.
        - ⇒ Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol or special DNA decontamination reagents.
        - ⇒ Pipette the Positive control at last.
        - ⇒ Repeat the PCR preparation with the new set of reagents.

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	**C–**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

REFERENCES

- Role of Chlamydia trachomatis in Miscarriage. Baud D, Goy G, Jaton K, Osterheld MC, Blumer S, Borel N, Vial Y, Hohlfeld P, Pospischil A, Greub G. Emerg Infect Dis. 2011 Sep;17(9):1630-5.
- Molecular Diagnosis of Genital Chlamydia trachomatis Infection by Polymerase Chain Reaction. Khan ER, Hossain MA, Paul SK, Mahmud MC, Rahman MM, Alam MA, Hasan MM, Mahmud NU, Nahar K. Mymensingh Med J. 2011 Jul;20(3):362-5.
- Chlamydia trachomatis prevalence in unselected infertile couples.Salmeri M, Santanocita A, Toscano MA, Morello A, Valenti D, La Vignera S, Bellanca S, Vicari E, Calogero AE. Syst Biol Reprod Med. 2010 Dec;56(6):450-6. Epub 2010 Sep 17.
- Urine-based testing for Chlamydia trachomatis among young adults in a population-based survey in Croatia: feasibility and prevalence. Božičević I, Grgić I, Židovec-Lepej S, Čakalo JI, Belak-Kovačević S, Štulhofer A, Begovac J. BMC Public Health. 2011 Apr 14;11:230.
- Frequency of Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma genitalium, Mycoplasma hominis and Ureaplasma species in cervical samples. Rodrigues MM, Fernandes PÁ, Haddad JP, Paiva MC, Souza Mdo C, Andrade TC, Fernandes AP. J Obstet Gynaecol. 2011;31(3):237-41.
- Chlamydia trachomatis prevalence in unselected infertile couples. Salmeri M, Santanocita A, Toscano MA, Morello A, Valenti D, La Vignera S, Bellanca S, Vicari E, Calogero AE. Syst Biol Reprod Med. 2010 Dec;56(6):450-6. Epub 2010 Sep 17.
- Prevalence of Chlamydia trachomatis: results from the first national population-based survey in France. Goulet V, de Barbeyrac B, Raherison S, Prudhomme M, Semaille C, Warszawski J; CSF group. Sex Transm Infect. 2010 Aug;86(4):263

*iQ5™ is a registered trademark of Bio-Rad Laboratories
* Rotor-Gene™ Technology is a registered trademark of Qiagen
* MX3005P® are trademarks of Agilent Technologies
*ABI® is trademarks of Applied Biosystems
* LineGeneK® a registered trademark of Bioer
* SmartCycler® is a registered trademark of Cepheid

Sacace Biotechnologies Srl
via Scalabrini, 44 – 22100 – Como – Italy Tel +390314892927 Fax +390314892926
mail: ***info@sacace.com*** *web:* ***www.sacace.com***