For Professional Use Only

Chlamydia trachomatis A,B,C Typing Real-TM

Handbook

Multiplex Real Time PCR kit for qualitative detection of Chlamydia trachomatis A, B, C types

REF B86-100FRT

REF TB86-100FRT

Σ 100

NAME

Chlamydia trachomatis A,B,C Typing 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 A,B,C Typing Real-TM PCR kit is an *in vitro* nucleic acid amplification test for multiplex detection of *Chlamydia trachomatis A, B, C* DNA in clinical materials (urogenital, rectal and pharyngeal swabs; conjunctival discharge; prostate gland secretion; and urine samples) by using 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 A, B, C detection by the multiplex polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using specific *Chlamydia trachomatis A, B, C* 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 during thermocycling. The real-time monitoring of the 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 A,B,C Typing Real-TM** PCR kit is a qualitative test that contains the Internal Control (IC). It 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 A,B,C Typing Real-TM** PCR kit uses “hot-start”, which greatly reduces 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.

***Chlamydia trachomatis A, B, C* genotypes are detected on the FAM (Green) channel**

***Chlamydia trachomatis* all genotypes are detected on the JOE (Yellow) channel**

***IC DNA* is detected on the ROX (Orange) channel**

MATERIALS PROVIDED

Module No.1: Real Time PCR kit (B86-100FRT)

Part N° 2 – "**Chlamydia trachomatis A,B,C Typing Real-TM** "

- **PCR-mix-1-FL**, 1,2 ml;
- **PCR-mix-2**, 2 x 0,3 ml;
- **TaqF Polymerase**, 2 x 0,03 ml;
- **Pos C+**, 0,2 ml;
- **Negative Control C-***, 1,2 ml;
- **Internal Control IC****, 1,0 ml;
- **DNA-buffer**, 0,5 ml;

Contains reagents for 110 tests.

Module No.2: Complete Real Time PCR test with DNA purification kit (TB86-100FRT)

Part N° 1 – "**DNA-sorb-A**":

- **Lysis Solution**, 2 x 15 ml;
- **Sorbent**, 2 x 1,0 ml;
- **Washing Solution**, 2 x 50 ml;
- **DNA-eluent**, 2 x 5 ml;
- **Transport medium** , 30 ml.

Contains reagents for 100 tests.

Part N° 2 – "**Chlamydia trachomatis A,B,C Typing Real-TM** "

- **PCR-mix-1-FL**, 1,2 ml;
- **PCR-mix-2**, 2 x 0,3 ml;
- **TaqF Polymerase**, 2 x 0,03 ml;
- **Pos C+**, 0,2 ml;
- **Negative Control C-***, 1,2 ml;
- **Internal Control IC****, 1,0 ml;
- **DNA-buffer**, 0,5 ml;

Contains reagents for 110 tests.

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

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

ADDITIONAL REQUIREMENTS

- 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 PCR-plate
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

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 (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.

WARNINGS AND PRECAUTIONS

RUO *For Research Use Only*

The user should always pay attention to the following:

- ⚠ Lysis Solution contains guanidine thiocyanate*. Guanidine thiocyanate is harmful if inhaled, or comes into contact with skin or if swallowed. Contact with acid releases toxic gas. (Xn; R: 20/21/22-36/37/38; S: 36/37/39).
- 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.

**** Only for Module No.2***

STORAGE INSTRUCTIONS

All components of the **Chlamydia trachomatis A,B,C Typing Real-TM** PCR kit (except for **TaqF Polymerase** and **PCR-mix-2**) are to be stored at the temperature 2–8 ºC when not in use. All components of the **Chlamydia trachomatis A,B,C Typing Real-TM** PCR kit are stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FL is to be kept away from light.

TaqF Polymerase and **PCR-mix-2** are to be stored at ≤ –16 °C

STABILITY

Chlamydia trachomatis A,B,C Typing 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. The shelf life of reagents before and after the first use is the same, unless otherwise stated. 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 A,B,C Typing Real-TM can analyze DNA extracted from:

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

The following isolation kit is recommended:

⇒ **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. 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)

SPECIMEN AND REAGENT PREPARATION (reagents supplied with the module no.2)

1. Lysis Solution and Washing Solution (in case of their storage at +2-8°C) should be warmed up to 60–65°C until disappearance of ice crystals. Prepare required quantity of 1.5 ml polypropylene tubes including one tube for Negative Control of Extraction.
2. Add to each tube 10 µl of Internal Control (Sacace IC is the same for all urogenital infectious Real Time kits) and 300 µl of Lysis Solution.
3. Add 100 µl of Samples to the appropriate tube.
4. Prepare Controls as follows:
    - add 100 µl of C– (Neg Control provided with the amplification kit) to the tube labeled Cneg.
5. Vortex the tubes and incubate for 5 min at 65°C. Centrifuge for 5-7 sec. If the sample is not completely dissolved it is recommended to re-centrifuge the tube for 5 min at a maximum speed (12000-16000 g.) and transfer the supernatant into a new tube for DNA extraction.
6. Vortex vigorously Sorbent and add 20 µl to each tube.
7. Vortex for 5-7 sec and incubate all tubes for 3 min at room temperature. Repeat this step.
8. Centrifuge all tubes for 30 sec at 5000g and using a micropipette with a plugged aerosol barrier tip, carefully remove and discard supernatant from each tube without disturbing the pellet. Change tips between the tubes.
9. Add 500 µl of Washing Solution to each tube. Vortex very vigorously and centrifuge for 30 sec at 10000g. Remove and discard supernatant from each tube.
10. Repeat step 9 and incubate all tubes with open cap for 5-10 min at 65°C.
11. Resuspend the pellet in 100 µl of DNA-eluent. Incubate for 5 min at 65°C and vortex periodically.
12. Centrifuge the tubes for 1 min at 12000g.

13. The supernatant contains DNA ready for amplification. 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

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

1. Prepare required quantity of reaction tubes for samples (N) and controls (N+2).
2. Prepare in the new sterile tube **10*N µl** of **PCR-mix-1-FL**, **5*N µl** of **PCR-mix-2** and **0,5*N µl** of **TaqF Polymerase.** Vortex and centrifuge briefly.
3. Add to each tube **15 µl** of **Reaction Mix** and **10 µl** of **extracted DNA**. Mix by pipetting.
4. Prepare for each panel 2 controls:
   - add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification);
   - add **10 µl** of **Pos C+** to the tube labeled C+ (PCR Positive Control);
5. Insert the tubes in the thermalcycler.

Amplification

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

Step	**Rotor type instruments[1]**			**Plate or modular type instruments[2]**		
	Temperature, °C	***Time***	***Cycles***	***Temperature, °C***	***Time***	***Cycles***
Hold	95	15 min	1	95	15 min	1
Cycling	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
Cycling 2	95	5 s	40	95	5 s	40
	60	**20 s (fluore-scence detection)**		60	**30 s (fluore-scence detection)**	
	72	15 s		72	15 s	

[1] For example Rotor-Gene™ 3000/6000/Q (Corbett Research, Qiagen)
[2] For example, SaCycler-96™ (Sacace), CFX/iQ5™ (BioRad); Mx3005P™ (Agilent), ABI® 7500 Real Time PCR (Applied Biosystems), SmartCycler® (Cepheid)

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

Fluorescence is detected at the 2nd step of Cycling 2 stage (60 °C) in FAM/Green, JOE/Yellow/HEX/Cy3 and ROX/Orange/Texas Red fluorescence channels.

INSTRUMENT SETTINGS

Rotor-type instruments (RotorGene 6000/Q)

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

Plate- or modular type instruments (SaCycler, iQ5, Mx300P, ABI 7500, SmartCycler)

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

- ***Chlamydia trachomatis A, B, C* types** amplification product is detected is in the **FAM/Green** fluorescence channel,
- ***Chlamydia trachomatis all genotypes*** amplification product is detected in the **JOE/HEX/Yellow** fluorescence channel,
- **Internal Control DNA** is detected in the **ROX/Orange** channel,

The results of the analysis are considered reliable only if the results obtained for both Positive and Negative Controls are correct.

Table 2. Results for controls

Control	Stage for control	Ct channel FAM/Green, JOE/Yellow/HEX	Ct channel ROX/Orange	Interpretation
C–	DNA extraction	Neg	Pos (< 33)	OK
NCA	Amplification	Neg	Neg	OK
C+	Amplification	Pos (< 33)	Pos (< 33)	OK

1. The sample is considered to be **positive** for ***Chlamydia trachomatis A, B, C* types** if its Ct value is detected in the results grid in the **FAM/Green** channel. Moreover, the fluorescence curve should cross the threshold line in the region of exponential fluorescence growth.
2. The sample is considered to be **negative** for ***Chlamydia trachomatis A, B, C* types** but to be **positive** for ***Chlamydia trachomatis other genotypes*** if its Ct value is not detected in the results grid in **FAM/Green** channel and detected in the **JOE/Yellow/HEX** with Ct less than 36.
3. The sample is considered to be **negative** for ***Chlamydia trachomatis A, B, C* types** if its Ct value is not detected in the results grid in **FAM/Green** channel and detected in the **ROX/Orange** channel (Internal Control) with Ct less than 36.

TROUBLESHOOTING

1. Weak or no signal of the IC (ROX/Orange 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
    - 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. Any signal on Fam(Green) and Joe(Yellow)/Hex/Cy3 channels 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
RUO	For Research Use Only	**NCA**	Negative Control of Amplification
	Store at	**C–**	Negative control of Extraction
	Manufacturer	**C+**	Positive Control of Amplification
	Consult instructions for use	**IC**	Internal Control
	Expiration Date		

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