CHLAMYDIA TRACHOMATIS DNA

Qualitative Real -Time PCR for detection of Chlamydia trachomatis

-for “in vitro” diagnostic use only-

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REF. CTDNA.CE
25/50/100/150 Tests

Chlamydia trachomatis DNA

A. INTENDED USE

The **Chlamydia trachomatis DNA** Real-Time PCR kit coded **CTDNA.CE** is intended for the qualitative detection of Chlamydia trachomatis DNA in human urethral/cervical swabs and urine with a simultaneous control of the extraction/amplification reaction through an **Internal Control (IC).**

Importante note: The CTDNA.CE kit it is able to detect the Swedish C.trachomatis variant.

B. INTRODUCTION

Chlamydia trachomatis (CT), an obligate intracellular bacterium, is the most common sexually transmitted infection worldwide. CT infection may cause urethritis, cervicitis and pelvic inflammatory disease in women. Than 50% of Chlamydia trachomatis infections are asymptomatic, they may remain undetected for extended periods of time. Untreated or undiagnosed cervical infections in females can ascend into the upper genital tract, causing pelvic inflammatory disease and ectopic pregnancy. Infertility in males and females can result from untreated or undiagnosed chlamydial infections.

Chlamydia trachomatis can be easily treated and cured with antibiotics. A single dose of azithromycin or a week of doxycycline (twice daily) are the most commonly used treatments.

CT contains a single chromosome of 1.043000 bp. The genome codes for approximately 875 proteins, not all of which are necessarily expressed. In addition to the chromosome, CT commonly posses an extrachromosomal genetic element (cryptic plasmid) of 7493 bp. The plasmid is very highly conserved, with less than 1% variation in nucleotide sequence.

Nucleic acid amplification techniques (NAATs) have surpassed cell culture and antigen detection for the diagnosis of Chlamydia trachomatis infections due to their enhanced sensitivities.

C. PRINCIPLE OF THE TEST

The CTDNA.CE Kit is based on a Real Time chemistry which uses specific Primers and Probes,

Chlamydia trachomatis DNA, recovered from the biological sample under investigation through an extraction step, is amplified using Real Time amplification system. The amplified product is detected using a fluorescent reporter dye probe specific for a Chlamydia Trachomatis highly repeated (1 to 10 times) genomic sequence.

Heterologous Internal Control (IC) serves as an Extractio/Amplification control for each individually processed specimen aiming to the identification of reaction inhibitors.

An High Positive control (CTRL-H) and a Low Positive control (CTRL-L) are supplied as controls of the PCR reaction.

D. COMPONENTS

The standard format of the product code CTDNA.CE contains reagents for 50 tests.

Component	Contents	CTDNA.CE 50 Reactions
A **CODED: ALL/MM5** **COLOR CODE: CLEAR**	Master mix	N° 1 Vial / 0.825 ml
B **CODED: CT/CB** **COLOR CODE: YELLOW**	Lyophilised Primers/Probes	N°2 vials (Dissolve with the volume of ALL/C indicated on the vial label)
C **CODED: ALL/C** **COLOR CODE: RED**	MG Water	N°2 vials /1.5 ml
NTC **CODED: ALL/NTC** **COLOR CODE: WHITE**	Negative Control	N°1 vials /1.5 ml
CTRL-H High Positive Control (10^4 Copies/ul) **CODED: CT/CTRL-H** **COLOR CODE: VIOLET**	Lyophilised Qualitative High positive	N° 8 vials (Dissolve with the volume of ALL/C indicated on the vial label)
CTRL-L Low Positive Control (10 Copies/ul) **CODED: CT/CTRL-L** **COLOR CODE: PINK**	Lyophilised Qualitative Low positive	N° 8 vials (Dissolve with the volume of ALL/C indicated on the vial label)
I.C. Internal Control **CODED: ALL/IC** **COLOR CODE: GREEN**	Lyophilised Internal Control	N°2 vials (Dissolve with the volume of ALL/C indicated on the vial label)
Package Insert	Instruction for Use	1

Important note: Upon request, Dia.Pro can supply reagents for 25, 100, 150 tests, as reported below**:**

1. Component A	n°1 vial/0.4 ml	n°2 vial/0.825 ml	n°3 vial/0.825 ml
2. Component B	n°1 vial	n°4 vial	n°6 vial
3. Component C	n°1 vial/1.5 ml	n°2 vial/1.5 ml	n°3 vial/1.5 ml
4. NTC	n°1 vial/1.5 ml	n°1 vial/1.5 ml	n°1 vial/1.5 ml
5. IC	n°1 vial	n°4 vial	n°6 vial
6. CTRL-H	n°4 vial	n°4 vial	n°6 vial
7. CTRL-L	n°4 vial	n°4 vial	n°6 vial
8.Pack. insert	n°1	n°1	n°1
Number of tests	**25**	**100**	**150**
Code	**CTDNA.CE.25**	**CTDNA.CE.100**	**CTDNA.CE.150**

E. STORAGE AND STABILITY

The kit CTDNA.CE must be stored at +2…8 °C. Once dissolved, Component B (coded CTDNA/CB) and Component IC (coded ALL/IC) are stable for 4 months at -20°C. Once dissolved components positive controls HIGH and LOW (coded CT/CTRL-HIGH, CT/CTRL-LOW) are stable for 2 weeks at -20°C. If the components are to be used only intermittently, they should be frozen in aliquots, repeated thawing and freezing should be avoided, Only one defreezing is allowed.

F. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (0.5 µl < volume <1000 µl)
2. DNA extraction kit
3. MG EtOH
4. Thermal Block
5. Microcentrifuge
6. Tube racks
7. Sterile filtered tip with aerosol barrier
8. Nuclease-Free Microtubes
9. 0,2 ml Microtubes or Pcr Microplates recommended from the Real-Time PCR instruments manufacturers
10. Disposable gloves, powder-free
11. Real-Time PCR Thermalcycler (*)
12. Absorbent paper tissues.
13. Vortex or similar mixing tools.
14. PBS

(*) Attention: A valid calibration of the pure dyes (Pure Spectra Component File) and of the background (Background Component File) must be done routinely.

G. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. The technical personnel must be deeply trained in the use of Real-Time thermalcyclers, in the manipulation of Molecular Biology reagents and skilled in the Real-Time PCR amplification protocols.
3. The kit has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
4. All the personnel involved in performing the assay have to wear protective laboratory clothes, powder-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
5. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
6. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents.
7. Components A and B are light sensitive. Protect them from strong light exposition.
8. Avoid vibration of the bench surface where the test is undertaken.
9. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
10. Do not interchange components between different lots of the kits. Moreover, components between kits of the same lot should not be interchanged.
11. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
12. Avoid cross-contamination between samples by using disposable tips and changing them after each sample.
13. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
14. Do not use the kit after the expiration date stated on the external container label.
15. Treat all specimens as potentially infective. All human urethral, cervical swabs and urine specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
16. Store and extract specimens separately from the other reagents and use a separate room for their handling
17. Dissolve the lyophilised reagents with the correct amount, stated in the labels with Component C (Coded: ALL/C) supplied in the kit.
18. Carry on all the working operations as quickly as possible maintaining the components on ice or in a cooling block.
19. The laboratory workflow must proceed in an unidirectional way, beginning in the Extraction Area and moving to the Amplification and Data Analysis Area. Do not return samples, equipment and reagents to the area where previous steps have been performed.
20. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
21. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from sample extraction procedures, has to be treated as potentially infective material and inactivated before waste. Do not put in contact the extraction waste with bleach.
22. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
23. Other waste materials generated (example: tips used for samples) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

H. SPECIMEN: PREPARATION AND RECOMMENDATIONS

1. Cervical and urethral swabs must be collected in a suitable transport medium which preserves nucleic acid from bacteria
2. For urine samples use the first 20 to 30 ml of the initial flush of urine, which is thought to contain the highest concentration of diagnostically relevant components
3. Using, for urine samples, polypropylene tubes without any addition of preservatives
4. The urethral swabs, cervical swabs and the urine samples must be pre-treated before DNA extraction according section M
5. Urine and swab samples must be transported and stored +2 / +8°C for a maximum period of 3 days. We recommend, for the optimal storage of samples, to split them in several aliquots (minimum volume 100 µl) and store them frozen at -20°C….-80°C for a maximum period of 30 day from sampling. Avoid repeated freezing / thawing cycles.
6. When using frozen samples, thaw the samples just before the extraction in order to avoid nucleic acid degradation
7. Samples have to be clearly identified with codes or names in order to avoid result misinterpretation.

I. PREPARATION OF COMPONENTS AND WARNINGS

Master Mix:

Component A. Ready to use. Mix well on vortex before use and centrifuge briefly to collect the whole volume.

WARNING: Component A is light sensitive. Protect it from strong light exposition.

Primers/Probes:

Component B.

- Centrifuge the vial at 11000 rpm for 1 min.
- Open carefully the vial cap avoiding powder dispersion.
- Dissolve homogenously the Lyophilized Component B with the volume of Component C (Code: ALL/C) indicated on the vial label.
- Keep it on the benchtop for at least 10 min at room temperature (15°C <RT< 25°C).
- Briefly vortex.

WARNING: Component B is light sensitive. Protect it from strong light exposition.

MG Water:

Component C. Ready to use.

Negative Control:

NTC. Ready to use.

Positive Controls:

Component CTRL-H.

- Centrifuge the vial at 11000 rpm for 1 min.
- Open carefully the vial cap avoiding powder dispersion.
- Dissolve homogenously the Lyophilized CTRL-H with the volume of Component C (Code: ALL/C) indicated on the vial label.
- Keep it on the benchtop for at least 10 min at room temperature (15°C <RT< 25°C).
- Briefly vortex.

Component CTRL-L.

- Centrifuge the vial at 11000 rpm for 1 min.
- Open carefully the vial cap avoiding powder dispersion.
- Dissolve homogenously the Lyophilized CTRL-L with the volume of Component C (Code: ALL/C) indicated on the vial label.
- Keep it on the benchtop for at least 10 min at room temperature (15°C <RT< 25°C)
- Briefly vortex

Internal Control:

I.C.

- Centrifuge the vial at 11000 rpm for 1 min.
- Open carefully the vial cap avoiding powder dispersion.
- Dissolve homogenously the Lyophilized I.C. with the volume of Component C (Code: ALL/C) indicated on the vial label.
- Keep it on the benchtop for at least 10 min at room temperature (15°C <RT< 25°C)
- Briefly vortex

L. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. **Micropipettes** have to be calibrated and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-5%.
2. **Extraction Device:** The CTDNA.CE Kit is intended to be used in combination only with QIAamp DNA Minikit Code.51306 (QIAGEN) and with Nucleospin Blood kit Code: 740951 (Macherey-Nagel). The end users must strictly follow the Instruction for use supplied by the manufacturers.
3. **Real-Time Thermocyclers.** The CTDNA.CE Kit is intended for the use in combination only with the Real Time Thermal cyclers ABI 7500, software SDS version 1.3.1 (Applied Biosystems), MX3000P, software MxPro version 4.01 (Stratagene) and CFX96, software CFX manager version 1.7 (Biorad).

The end users must strictly follow the Instruments Instruction for use supplied by the manufacturers.

M. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that on the bottom of the Lyophilized components vials is present a well formed aggregate. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box.
3. Dissolve the Lyophilized Components with the appropriate amount of Component C (Molecular Grade water) as described in the proper section (I).
4. Turn the Thermalcyclers on, check settings and be sure to use the right assay protocol.
5. Follow strickly the Instruments Manual supplied by the manufacturers for the correct setting of the Real-Time Thermalcyclers.
6. Check that the micropipettes are set to the required volume.
7. Check that all the other equipment is available and ready to use.
8. In case of problems, do not proceed further with the test and advise the supervisor.

N. PRE-TREATMENT OF THE SAMPLE

Urine

- Centrifuge 4500prm for 10 minutes
- Discard the supernatants and dissolve the pellet in the correct amount of sterile PBS depending on the size of the pellet obtained (0.5-2ml).
- Proceed to DNA extraction

Swabs

- Centrifuge 4500prm for 10 minutes
- Discard the supernatants and dissolve the pellet in the correct amount of sterile PBS depending on the size of the pellet obtained (0.5-2ml).
- Proceed to DNA extraction

O. ASSAY PROCEDURE

The assay has to be carried out according to what reported below.

O.1 DNA extraction

The extraction step of the Chlamydia trachomatis genomic DNA has to be carried out exclusively in combination with the following kits:

Material	Description	Kit code	manufacturer
Urethral/ Cervical swabs and Urine	QIAamp DNA mini kit®	51306	Qiagen™
Urethral/ Cervical swabs and Urine	Nucleospin Blood	740951	MN™

Extraction of Chlamydia trachomatis genomic DNA from the **urine** specimen has to be carried out by the end-user, according to the Manufacturer's instructions, with the following kits:

- **QIAamp DNA Mini Kit (QIAGEN)**

Important Notes: The "DNA Purification from Tissue" Protocol described in the Manufacturer's Instruction has to be applied with the following modifications:

1. *Start the protocol from step n°2 using as sample 200 ul of the resuspended pellet described in section M (pre-treatment of the sample) instead of the Tissue sample.*
2. *In the step n° 11 use 100 ul of Eluition Buffer instead of 200 ul.*

- **NucleoSpin Blood Kit (Macherey-Nagel)**

Important Note: Follow the "Standard protocol for DNA purification from whole blood" (Protocol 5.1) described in the Manufacturer's Instruction with the following modifications:

1. *Start the protocol from step n°1 using as sample 200 ul of the resuspended urine pellet described in the section N (pre-treatment of the sample).*

Extraction from **swab** specimen has to be carried out by the end-user, according to the Manufacturer's instructions, with the following kits:

- **QIAamp DNA Mini Kit (QIAGEN)**

Important Notes: The "DNA Purification from Tissue" Protocol described in the Manufacturer's Instruction has to be applied with the following modifications:

1. *Start the protocol from step n°2a using as sample 200 ul of the resuspended pellet described in the section M (pre-treatment of the sample) instead of the Tissue sample.*
2. *In the step n° 11 use 50 ul of Eluition Buffer instead of 200 ul.*

- **NucleoSpin Blood Kit (Macherey-Nagel)**

Important Note: Follow the "Standard protocol for DNA purification from whole blood" (Protocol 5.1) described in the Manufacturer's Instruction with the following modifications:

1. *Start the protocol from step n°1 using as sample 200 ul of the resuspended urine pellet described in the section N (pre-treatment of the sample).*
2. *In the step n° 6 "Elute Highly pure DNA" use 50 ul of Eluition Buffer instead of 100 ul.*

The DNA collected from the samples, not used in the run has to be stored frozen adequately (-20°C….-80°C).

Important note: *The I.C. of the* CTDNA.CE Kit *can be used in the isolation procedure as extraction control.*
The Internal Control Ct value for the negative samples is used to evaluate if the DNA extraction procedure has been performed correctly (see section R).

For this application add 10 µl of I.C. to the lysis buffer and sample mixture and proceed following the instruction manual supplied by the manufacturer of the Extraction Kit.

O.2 Setting up of the reaction

CTDNA.CE kit is intended to be used exclusively in combination with ABI 7500, software SDS version 1.3.1 (Applied Biosystems), MX3000P, software MxPro version 4.01 (Stratagene) and CFX96, software CFX manager version 1.7 (Biorad).

O.2.1 Preparing the PCR

Important: *An example of dispensation scheme is reported in Section P. Please, refer to it before starting to read the instructions here below.*

- Prepare the components as described in Section I;
- Prepare the required number of reaction tubes or a 96-well reaction plate for the samples under evaluation and for the Positive controls (prepared as described in section I).

Important note: *Use only optical tubes or microplates suggested by the Real-Time thermalcyclers manufacturers.*

- Consider that the samples, if possible, should be tested in duplicate;
- Include at least 1 tube/well for the NTC (negative control)
- Prepare the **Amplification Mix** for **Samples, NTC and positive controls (CTRL-H, CTRL-L)** as table below:

Preparation of the Amplification Mix

(I.C. as Amplification Control)

Number of Reactions		x1	x12
A	Master mix	12,5 µl	150 µl
B	Primers/probes	2 µl	24 µl
I.C.	Internal Control	0,5 µl	6 µl
Tot vol.		**15 µl**	**180 µl**

Important note: *If the Internal Control was added during the DNA isolation procedure, prepare the* ***Amplification Mix*** *for the* ***Sample,*** **NTC and positive controls (CTRL-H, CTRL-L),** *as described in the table below:*

Preparation of the Amplification Mix

(I.C. as Extraction/Amplification control)

Number of Reactions		x1	x12
A	Master mix	12,5 µl	150 µl
B	Primers/probes	2 µl	24 µl
C	MG Water	0,5 µl	6 µl
Tot vol.		**15 µl**	**180 µl**

O.2.2 Amplification procedure

- Dispense 15 ul of the amplification mix in each reaction tube or microplate well
- Add 10 ul of the **Samples, NTC, CTRL-H and CTRL-L** to the reaction tubes.
- Close firmly the reaction tubes
- Centrifuge briefly the reaction tubes at 2000 rpm
- Don't leave the reaction tubes at room temperature (RT) for more than 30 minute and at light exposure (cover the tubes).
- Load the reaction tubes in the Real-Time Thermalcycler Thermoblock Holder.
- After the setting operations described in the Sections O3 (Instrument Programming) start the Thermalcycler run.

Important note: The Components Lyophilized after dissolution in Component C (MG water) are stable no more than 3 hours kept in ice or at 2°…8° °C.
The not used volume of Component B, CTRL-H, CTRL-L and I.C. can be freeze at -20°C and used as described in Section E.

O.3 Instrument programming

For programming the instrument refer to the Instrumentation Instruction Manual provided by the manufacturers.
Important Note: For Mx3000P set "Filter set gain settings" : ROX = x1, FAM = x8, JOE = x1. (see MxPro™ QPCR Software Instruction Manual, p.41)

O.3.1 Thermal Profile

The thermal profile is reported in the table below:

Step	Cycle	Temp.	Time
1	1	50°C	2 min
2	1	95°C	10 min
3	50	95°C	15 sec
		60°C **(*)**	1 min

IMPORTANT NOTE: (*) step for the real time data collection
WARNING: Keep attention to set up the Real-Time Thermacycler with the correct Thermal Profile following the Instruments Manual supplied by the manufacturer.

O.3.2 Selection of the Detectors

Detectors have to be selected according to what described in the table below and in agree with the Instruction manuals of the Real-Time thermal cyclers used.

Detectors	CT	Internal Control (IC)	Passive Reference
Instrument	**Fluorophore**	**Fluorophore**	
ABI 7500 SDS	FAM - none	JOE - none	ROX
STRATAGENE Mx3000P®	FAM	JOE	ROX
BIORAD CFX96®	FAM	VIC	-

According to the Instruction Manual of the Real-Time thermal cycler suggested (ABI 7500 Applied Biosystems, Mx3000P Stratagene, BioRad CFX96,) choose the sample type and load the Detectors as reported in the table below:

Sample Type	CTRL	SAMPLE (Unknown)	NTC
	Detectors	**Detectors**	**Detectors**
ALL INSTRUMENTS	CT IC (optional)	CT IC	CT IC

WARNING: Keep attention to program the Real-Time Thermal cycler with the correct settings following the Instrument Manual supplied by the manufacturer.

P. ASSAY SCHEME
An example of dispensation scheme for Qualitative Analysis is reported below:

Microplate or tubes

	1	2	3	.	.
A	**CTRL-H** 10^4copies/ µl	Sample 6			
B	**CTRL-L** 10copies/ µl	Sample 7			
C	NTC	Sample 8			
D	Sample 1	Sample 9			
E	Sample 2	Sample 10			
F	Sample 3	Sample 11			
G	Sample 4	Sample 12			
H	Sample 5	Sample 13			

Legend: NTC = Negative Control CTRL-H, CTRL-L = Chlamydia trachomatis DNA Positive Controls, Sample 1,2,3 = Samples under evaluation.

Q. INTERNAL QUALITY CONTROL

Q.1 Pre - Analysis Settings

Before starting the analysis:

- Set the "Baseline" (the background fluorescence level) as reported here below:

"Baseline"	
ABI™PRISM® 7500 SDS	**Auto Baseline**
BIORAD™ CFX96®	Auto calculated Baseline
STRATAGENE™ MX3000P®	**Adaptive Baseline (not use Mx4000 v1.00 to v3.00 algorithm)**

- Set manually the FAM/JOE/VIC fluorescence "**Threshold**"

FAM fluorescence "Threshold"	
ABI™PRISM® 7500 SDS	**0.13**
STRATAGENE™ MX3000P®	**0.13**
BIORAD™ CFX96®	**300**

JOE/VIC fluorescence "Threshold"	
ABI™PRISM® 7500 SDS	**0.08**
STRATAGENE™ MX3000P®	**0.02**
BIORAD™ CFX96®	**150**

Q.2 Data analysis
A check is carried out on the High/Low Positive Controls any time the kit is used in order to verify whether their Ct values are as expected and reported in the table below.

ABI™PRISM® 7500 SDS - STRATAGENE™ Mx3000P® - BIORAD™ CFX96®	
Check FAM	**Requirements**
CTRL-H	22 ≤ Ct (Threshold Cycle) <26
CTRL-L	32 ≤ Ct (Threshold Cycle) <36

R. INTERPRETATION OF THE RESULTS AND TROUBLESHOOTING
For each samples FAM fluorescence (positive/negative Ct value) and Internal Control JOE fluorescence are assumed to validate CT DNA detection as described in the table below:

C trachomatis FAM	Internal Control JOE	Assay Result
SAMPLE POSITIVE	20 < Ct < 40	CORRECT
	Ct > 40 or undetermined	CORRECT*
SAMPLE NEGATIVE	20 < Ct < 40	CORRECT
	Ct > 40 or undetermined	INVALID**

*High Initial concentration of C.trachomatis DNA in the sample (Positive FAM Signal) can lead to REDUCED or ABSENT Fluorescent Signal for Internal Control I.C. due to the reagents Competition.

** Problems may be occurred during the amplification step (inefficient or absent amplification) or during the extraction step (presence of inhibitors or initial sample containing an insufficient number of cells) leading to an incorrect result. The test procedure must be repeated starting from the Extraction step using a fresh sample coming from the patient.

The results obtained with this product must be interpreted taking consideration the clinical symptoms and the other laboratory parameters related to the patient conditions.

The following results are possible:

Troubleshooting table

	FAM	JOE	Result	CHECK
SAMPLE unknown	+	+/-	CORRECT RESULT *Positive*	***IMPORTANT***: High Initial concentration of CT DNA (Positive FAM Signal) can lead to REDUCED or ABSENT Fluorescent Signal of Internal Control I.C. due to the reagents Competition.
SAMPLE unknown	-	-	ATTENTION ! POSSIBILITY OF: Inhibition, error in the procedure or misfunctioning of the Instruments	1. that the components have been prepared correctly 2. that no mistake has been done in the assay procedure; 3. that the selected detection dyes are corrected FAM for the CT detection and JOE for the I.C. detection; 4. that the Analysis has been run with the correct Instrument settings; 5. that the kit has been stored correctly; 6. that no potential PCR inhibitors have been contaminated the tube 7. that the Extraction procedure have been executed correctly;
SAMPLE unknown	-	+	CORRECT RESULT *Negative*	
CTRL-H/CTRL-L	+	+/-	CORRECT RESULT	***IMPORTANT***: High Initial concentration of CT DNA (Positive FAM Signal) can lead to REDUCED or ABSENT Fluorescent Signal of Internal Control I.C. due to the reagents Competition.
CTRL-H/CTRL-L	-	-	ATTENTION ! POSSIBILITY OF: Error in the pipetting or in the procedure	1. that the components have been prepared correctly 2. that no mistake has been done in the assay procedure; 3. That the selected detection dyes are corrected FAM for the Chlamydia trachomatis detection and JOE for the I.C. detection; 4. that the Analysis has been run with the correct Instrument settings; 5. that the kit has been stored correctly; 6. that no potential PCR inhibitors have been contaminated the tube
CTRL-H/CTRL-L	-	+	ATTENTION ! POSSIBILITY OF: Error in the pipetting or in the procedure	1. that the components have been prepared correctly 2. that no mistake has been done in the assay procedure; 3. That the selected detection dyes are corrected FAM for the Chlamydia trachomatis detection and JOE/VIC for the I.C. detection; 4. that the Analysis has been run with the correct Instrument settings; 5. that the kit has been stored correctly;
NTC	-	+	CORRECT RESULT	
NTC	+	+/-	ATTENTION ! POSSIBILITY OF: Contamination	1. that the components have been prepared correctly 2. that no mistake has been done in the assay procedure; 3.that the work space and Instruments are decontaminated at regular intervals; 4. that the kit has been stored correctly;

Important notes:

1. *Interpretation of results should be done under the supervision of the responsible of the laboratory to reduce the risk of judgment errors and misinterpretations.*
2. *When test results are transmitted from the laboratory to an informatics centre, attention has to be paid to avoid erroneous data transfer.*

If one of more of the problems described in the table above happen, after checking, report any residual problem to the supervisor for further actions.

S. PERFORMANCES

Evaluation of Performances has been conducted in accordance to what reported in the Internal Technical Specifications or ITS.
The performance evaluation was carried out in DiaPro's laboratories on materials supplied by the reference clinical lab.

S.1 ANALYTICAL SENSITIVITY

Analytical sensitivity may be expressed for qualitative methods as **limit of detection**.
Limit of detection (LOD): it is the lowest amount of target that can be detected by a test system with a stated probability.
For the NAT tests it is expressed as the smallest concentration of the **analyte** that tested in multiple repetitions gives a positive results.
The **limit of detection (LOD)** is determined by testing serial dilutions containing known concentrations of the analyte.
The **LOD** is the lowest concentration of analyte that can be consistently detected (e.g. in $\geq$ 95% of samples under routine laboratory conditions).
For the kit CTDNA.CE the **LOD** has been determined by testing 1:2 serial dilutions (8 replicates for three different runs) of a plasmid carrying the viral target sequence.
The results were analized by a **Probit** analysis, to determine the detection limit at 95%.
The LOD has been confirmed on CFX96RTS testing the corresponding dilution in 24 replicates.

The results are the following:

LOD Limit of Detection (p=0.05)	
ABI™PRISM® 7500 SDS	**0.53 copies/ µl**
STRATAGENE™ MX3000P®	**0.53 copies/ µl**
BIORAD CFX96® RTS	**0.53 copies/ µl**

Important Note*: The target is a sequence of Plasmid Cryptic that is repeated 1-10 fold in the genome of Chlamydia trachomatis.*

S.2 ANALITYCAL SPECIFICITY

The Analytical specificity is the ability of the method to detect only the target DNA sequence.
The analytical specificity of CTDNA assay has been studied as follow:

1. The primer/probe Set has been choose analysing the genome target sequence with an appropriate software (LionSoft v.1.0 supplied by Biotools and Primer Express v.3.0" supplied by Applied Biosystem Inc.).
2. The primer/probe Set and the target genome sequence has been controlled by the "BLAST" software, in order to check if any of the nucleotide sequences deposited in the worldwide genomic banks has any homology with Chlamydia trachomatis, and by the "ClustalX" software, in order to compare the genome target sequences of the different genotypes of C.trachomatis.
3. The specificity was improved through the selection of stringent reaction conditions.
4. Genomic DNA isolated from bacteria potential interfering organisms with Chlamydia trachomatis were obtained from American Type Culture Collection (ATCC) and Vircell and Quality Control for Molecular Diagnostics (QCMD) and were tested

The results are reported in the following table:

Organism	Results
Chlamydia pneumoniae	negative
Chlamydia psittaci	negative
Candida albicans	negative
Candida glabrata	negative
Mycoplasma hominis	negative
Ureaplasma urealyticum	negative
Acinetobacter spp	negative
Escherichia coli	negative
Klebsiella pneumoniae	negative
Streptococcus pyogenes	negative
Proteus mirabilis	negative
Neisseria gonorrhoeae	negative

S.3 DIAGNOSTIC SPECIFICITY AND SENSITIVITY

S.3.1 Diagnostic Specificity:
Diagnostic specificity is the probability that the device gives a negative result in the absence of the target marker. So the **true negative** sample is a specimen known to be negative for the target marker and correctly classified by the device.
This parameter was studied by examining 49 C. trachomatis DNA negative samples (30 urine and 19 cervical swabs) using the NicleoSpin Tissue extraction method.
In addition, 9 negative urine and 1 negative cervical swab samples have been purified with the NucleoSpin Blood kit.

TRUE NEGATIVES_Tissue MN	49
TRUE NEGATIVES_ Blood MN	10
FALSE POSITIVES	0
TOTAL SAMPLES	59
SPECIFICITY %	**100**

On the basis of the results obtained Diagnostic Specificity of the system has been calculated ≥99%.

S.3.2 Diagnostic Sensitivity
Diagnostic sensitivity is the probability that the device gives a positive result in the presence of the target marker. So, the **true positive** sample is a specimen known to be positive for the target marker and correctly classified by the device.
For the kit CTDNA.CE this parameter was studied by examining 12 C. trachomatis DNA positive cervical swabs samples and 7 C. trachomatis DNA positive urine samples using the NucleoSpin Tissue extraction method.
In addition, 6 C. trachomatis DNA positive samples (4 urine and 2 cervical swab) have been purified with the NucleoSpin Blood kit.

TRUE POSITIVES_Tissue MN	12
TRUE POSITIVES_Blood MN	6
FALSE NEGATIVES	0
TOTAL SAMPLES	18
SENSITIVITY %	**100**

On the basis of the results obtained Diagnostic Sensitivity of the system has been calculated in the 100%.

Diagnostic Sensitivity	100 %
Diagnostic Specificity	> 99.5 %

S.4 PRECISION
Precision shows the degree of the system's reliability. Every measurement procedure has an inherent random variation called "random error". Random error does not have a number value but it is determined by dispersion of measurement as standard deviation (DevST) and coefficient variation (CV%). Usually precision of an assay refers to the agreement between replicate measurements of the same material.
For the kit CTDNA.CE, **precision** was expressed as intra-assay variability and inter-assay variability. CTRL-H and CTRL-L in 8 replicates were tested in the same run (intra-assay) and in three different runs (inter-assay).
On the basis of the results obtained intra and inter-assay variability were then calculated.

In absence of established International parameters we have identified the following value of acceptability for the CTDNA.CE Kit:
Intra-Assay Coefficient Variation (CV%) ≤ 10%.
Inter-Assay Coefficient Variation (CV%) ≤ 10%.

T. LIMITATIONS
The user of this kit is advised to carefully read and understand this package insert. Strict adherence to the protocol is necessary in order to obtain reliable test results. In particular, accurate sample and reagent pipetting, application of a correct workflow along with careful programming of thermocycling steps are essential for accurate and reproducible C.trachomatis DNA detection.
It is recommended that confidentiality, appropriate counselling and medical evaluation be considered as an essential aspect of the testing sequence.

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5. Symbols

LEGENDA			
REF	Product code		Storage temperature
IVD	In Vitro Diagnostic Device		See use instructions
LOT	Lot number		Manufacturer
	Expiry date		Number of tests
CE	CE conformity mark		Date of manufacturing

All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
Dia.Pro Diagnostic Bioprobes Srl
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