

# 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)**.

The kit has been adapted for the use on the Real-Time Thermocyclers ABI 7500 Sequence Detection System® (Applied Biosystems™\*), or MiniOpticon® (Biorad™\*\*) or MX3000P® (Stratagene™\*\*\*).

\* Applied Biosystems is a registered trademark and ABI PRISM® is a trademark of Applied Biosystems Corporation or its subsidiaries in the US and/or certain other countries.

\*\* Biorad is a registered trademark.

\*\*\*Stratagene is a registered trademark.

### 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 poses 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<br>50 Reactions                        |
|---|---|---|
| <b>A</b><br>CODED: ALL/MM<br>COLOR CODE: LIGHT BLUE   | Master mix                                  | N°2 Vials /<br>0.350 ml                         |
| <b>B</b><br>CODED: CT/CB<br>COLOR CODE: YELLOW  | Lyophilised<br>Primers/Probes               | N°2 Vials<br>(dissolve with 60<br>ul of ALL/C)  |
| <b>C</b><br>CODED: ALL/C<br>COLOR CODE: GRAY  | MG Water                                    | N°1 vial /1.5 ml                                |
| <b>NTC</b><br>CODED: ALL/NTC<br>COLOR CODE: WHITE   | Negative<br>Control                         | N°1 vial /1.5 ml                                |
| <b>CTRL-H</b><br>High Positive Control<br>(10 <sup>4</sup> Copies/ul)<br>CODED: CT/CTRL-H<br>COLOR CODE: VIOLET | Lyophilised<br>Qualitative<br>High positive | N°2 Vials<br>(dissolve with 30<br>ul of ALL/C)  |
| <b>CTRL-L</b><br>Low Positive Control<br>(10 Copies/ul)<br>CODED: CT/CTRL-L<br>COLOR CODE: PINK                 | Lyophilised<br>Qualitative<br>Low positive  | N°2 Vials<br>dissolve with 30<br>ul of ALL/C)   |
| <b>I.C.</b><br>Internal Control<br>CODED: CT/IC<br>COLOR CODE: GREEN  | Lyophilised<br>Internal Control             | N°2 Vials<br>(dissolve with<br>300 ul of ALL/C) |
| Package Insert  | Instruction<br>for Use                      | N°1   |

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

|                        |                    |                     |                     |
|------------------------|--------------------|---------------------|---------------------|
| 1. Component A         | n°1 vial/0.350 ml  | n°4 vials/0.350 ml  | n°6 vials/0.350 ml  |
| 2. Component B         | n°1 vial           | n°4 vials           | n°6 vials           |
| 3. Component C         | n°1 vial/1.5 ml    | n°1 vial/1.5 ml     | n°1 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 vials           | n°6 vials           |
| 6. CTRL-H              | n°1 vial           | n°4 vials           | n°6 vials           |
| 7. CTRL-L              | n°1 vial           | n°4 vials           | n°6 vials           |
| 8. Pack. insert        | n°1                | n°1                 | n°1                 |
| <b>Number of tests</b> | <b>25</b>          | <b>100</b>          | <b>150</b>          |
| <b>Code</b>            | <b>CTDNA.CE.25</b> | <b>CTDNA.CE.100</b> | <b>CTDNA.CE.150</b> |

#### E. 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.

#### F. 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.

#### G. 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.

#### H. 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 for 1 min at 2000 rpm.
- 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 and in the Section D table.
- Keep it on the benchtop for at least 10 min at room temperature ( 15°C <RT< 25°C)

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

##### MG Water :

Component C. Ready to use.

### Negative Control :

N.T.C. Ready to use.

### Positive Controls:

#### Component CTRL-H.

- Centrifuge the vial for 1 min at 2000 rpm.
- 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 and in the Section D table.
- Keep it on the benchtop for at least 10 min at room temperature ( 15°C <RT< 25°C)

#### Component CTRL-L.

- Centrifuge the vial for 1 min at 2000 rpm.
- 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 and in the Section D table.
- Keep it on the benchtop for at least 10 min at room temperature ( 15°C <RT< 25°C)

### Internal Control:

I.C.

**Important Note:** Dissolve with 300 ul of Component C ALL/C

- Centrifuge the vial for 1 min at 2000 rpm.
- Open carefully the vial cap avoiding powder dispersion.
- Dissolve homogenously the Lyophilized I.C. with the volume of Component C (Code: ALL/C) indicated in the Section D table.
- Keep it on the benchtop for at least 10 min at room temperature ( 15°C <RT< 25°C)

### I. 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 Nucleospin Tissue kit Code: 740952 (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 (Applied Biosystems), and MX3000P (Stratagene) and MiniOpticon (Biorad) thermocyclers. The end users must strictly follow the Instruments Instruction for use supplied by the manufacturers.

### L. 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 (H).
4. Turn the Thermalcyclers on, check settings and be sure to use the right assay protocol.
5. Follow strictly 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.

### M. PRE-TREATMENT OF THE SAMPLE

#### Urine

- Centrifuge 4500rpm 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 4500rpm 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

### N. ASSAY PROCEDURE

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

#### N.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/<br>Cervical swabs<br>and Urine | QIAamp DNA<br>mini kit® | 51306    | Qiagen™      |
| Urethral/<br>Cervical swabs<br>and Urine | NucleoSpin<br>Tissue    | 740952   | 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°2a 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 Elution Buffer instead of 200 ul.

#### • **NucleoSpin Tissue Kit (Macherey-Nagel)**

**Important Note:** Follow the " Standard protocol for human or animal tissues and cultured cells" (Protocol 5.1) described in the Manufacturer's Instruction with the following modifications:

1. Start the protocol from step n°2 "Pre-Lysis" using as sample 200 ul of the resuspended pellet described in the section M (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<sup>o</sup> 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<sup>o</sup> 11 use 50 ul of Elution Buffer instead of 200 ul.

• **NucleoSpin Tissue Kit (Macherey-Nagel)**

**Important Note:** Follow the “ Standard protocol for human or animal tissues and cultured cells” (Protocol 5.1) described in the Manufacturer’s Instruction applying the following modifications:

1. Start the protocol from step n<sup>o</sup> 2 “Pre-Lysis” using as sample 200 ul of the resuspended pellet described in the section M (pre-treatment of the sample).
2. In the step n<sup>o</sup> 8 “ Elute Highly pure DNA” use 50 ul of Elution 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 Q).

**For this application add 5 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.**

**N.2 Setting up of the reaction**

CTDNA.CE kit is intended to be used exclusively in combination with ABI 7500 standard (Applied Biosystem), MiniOpticon (Biorad) and MX3000P (Stratagene).

**N.2.1 Preparing the PCR**

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

- Prepare the components as described in Section H;
- 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 H).

**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           |
|---------------------|------------------|--------------|---------------|
| <b>A</b>            | Master mix       | 12,5 µl      | 150 µl        |
| <b>B</b>            | Primers/probes   | 2 µl         | 24 µl         |
| <b>I.C.</b>         | Internal Control | 0,5 µl       | 6 µl          |
| <b>Tot vol.</b>     |                  | <b>15 µl</b> | <b>180 µl</b> |

**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           |
|---------------------|----------------|--------------|---------------|
| <b>A</b>            | Master mix     | 12,5 µl      | 150 µl        |
| <b>B</b>            | Primers/probes | 2 µl         | 24 µl         |
| <b>C</b>            | MG Water       | 0,5 µl       | 6 µl          |
| <b>Tot vol.</b>     |                | <b>15 µl</b> | <b>180 µl</b> |

**N.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 M5 (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 split in aliquots and kept frozen at -20°C. The aliquots must be used within seven (7) days from the freezing day.

### N.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, HEX/JOE = x1. (see MxPro™ QPCR Software Instruction Manual, p.41)

#### N.3.1 Thermal Profile

The thermal profile is reported in the table below:

| Step | Cycle | Temp.    | Time   |
|------|-------|----------|--------|
| 1    | 1     | 50°C     | 2 min  |
| 1    | 1     | 95°C     | 10 min |
| 2    | 45    | 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.

#### N.3.2 Selection of the Detectors

Following the Instruction manuals for the Real-Time thermacyclers suggested (ABI 7500, Biorad Miniopticon and MX3000P Stratagene) select the Detectors reported in the table here below:

| Detection               | Reporter | Quencher        |
|-------------------------|----------|-----------------|
| Chlamydia trachomatis   | FAM      | Non Fluorescent |
| Internal Control (I.C.) | JOE/HEX  | Non Fluorescent |
| Passive Reference       | ROX      | Not Present     |

WARNING: Keep attention to set up the Real-Time Thermacycler with the correct settings following the Instruments Manual supplied by the manufacturer.

### O. ASSAY SCHEME

An example of dispensation scheme for Qualitative Analysis is reported below:

#### Microplate or tubes

|   | 1                                    | 2         | 3 | 4 | 5 |
|---|--------------------------------------|-----------|---|---|---|
| A | CTRL-H<br>10 <sup>4</sup> copies/ µl | Sample 6  |   |   |   |
| B | CTRL-L<br>10 copies/ 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.

### P. INTERNAL QUALITY CONTROL

#### P.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™MiniOpticon®  | Global Minimum   |
| STRATAGENE™ MX3000P® | Adaptive Baseline<br>(not use Mx4000 v1.00 to v3.00 algorithm) |

- Set manually the FAM/JOE/HEX fluorescence "Threshold"

| FAM fluorescence "Threshold" |      |
|------------------------------|------|
| ABI™PRISM® 7500 SDS          | 0.13 |
| BIORAD™ MiniOpticon®         | 0.06 |
| STRATAGENE™ MX3000P®         | 0.13 |

| JOE/HEX fluorescence "Threshold" |      |
|----------------------------------|------|
| ABI™PRISM® 7500 SDS              | 0.13 |
| BIORAD™ MiniOpticon®             | 0.02 |
| STRATAGENE™ MX3000P®             | 0.02 |

#### P.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.

| Check FAM | Requirements                  |
|-----------|-------------------------------|
| CTRL-H    | 22 ≤ Ct (Threshold Cycle) <26 |
| CTRL-L    | 32 ≤ Ct (Threshold Cycle) <36 |

### Q. INTERPRETATION OF THE RESULTS AND TROUBLESHOOTING

For each samples FAM fluorescence (positive/negative Ct value) and Internal Control JOE/HEX fluorescence are assumed to validate CT DNA detection as described in the table below:

| C trachomatis FAM | Internal Control JOE/HEX | Assay Result |
|-------------------|--------------------------|--------------|
| SAMPLE POSITIVE   | +                        | CORRECT      |
|                   | -                        | CORRECT*     |
| SAMPLE NEGATIVE   | 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/<br>HEX | Result  | CHECK  |
|---------------------------|-----|-------------|---|--|
| SAMPL<br>E<br>unknown     | +   | +/-         | CORRECT<br>RESULT<br><u>Positive</u>  | <b>IMPORTANT:</b> 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.  |
| SAMPL<br>E<br>unknown     | -   | -           | ATTENTION !<br><br>POSSIBILITY<br>OF:<br><br>Inhibition,<br>error in the<br>procedure or<br>misfunctioning<br>of the<br>Instruments | 1. that the components have been prepared correctly<br>2. that no mistake has been done in the assay procedure;<br>3. that the selected detection dyes are corrected<br>FAM for the CT detection and JOE/HEX for the I.C. detection;<br>4. that the Analysis has been run with the correct Instrument settings;<br>5. that the kit has been stored correctly;<br>6. that no potential PCR inhibitors have been contaminated the tube<br>7. that the Extraction procedure have been executed correctly; |
| SAMPL<br>E<br>unknown     | -   | +           | CORRECT<br>RESULT<br><u>Negative</u>  |  |
| CTRL<br>-<br>H/CT<br>RL-L | +   | +/-         | CORRECT<br>RESULT   | <b>IMPORTANT:</b> 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<br>-<br>H/CT<br>RL-L | -   | -           | ATTENTION !<br><br>POSSIBILITY<br>OF:<br><br>Error in the<br>pipetting or in<br>the procedure                                       | 1. that the components have been prepared correctly<br>2. that no mistake has been done in the assay procedure;<br>3. That the selected detection dyes are corrected<br>FAM for the Chlamydia trachomatis detection and JOE/HEX for the I.C. detection;<br>4. that the Analysis has been run with the correct Instrument settings;<br>5. that the kit has been stored correctly;<br>6. that no potential PCR inhibitors have been contaminated the tube  |
| CTRL<br>-<br>H/CT<br>RL-L | -   | +           | ATTENTION !<br><br>POSSIBILITY<br>OF:<br><br>Error in the<br>pipetting or in<br>the procedure                                       | 1. that the components have been prepared correctly<br>2. that no mistake has been done in the assay procedure;<br>3. That the selected detection dyes are corrected<br>FAM for the Chlamydia trachomatis detection and JOE/HEX for the I.C. detection;<br>4. that the Analysis has been run with the correct Instrument settings;<br>5. that the kit has been stored correctly;   |
| NTC                       | -   | +           | CORRECT<br>RESULT   |  |

|     |   |     |  |  |
|-----|---|-----|--|--|
| NTC | + | +/- | ATTENTION !<br><br>POSSIBILITY<br>OF:<br><br>Contamination | 1. that the components have been prepared correctly<br>2. that no mistake has been done in the assay procedure;<br>3. that the work space and Instruments are decontaminated at regular intervals;<br>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 or more of the problems described in the table above happen, after checking, report any residual problem to the supervisor for further actions.

### R. 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.

#### R.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 analyzed by a **Probit** analysis, to determine the detection limit at 95%.

The results are the following:

| LOD Limit of Detection (p=0.05) |                 |
|---------------------------------|-----------------|
| ABI™PRISM® 7500 SDS             | 0.53 copies/ µl |
| BIORAD™ MiniOpticon®            | 0.53 copies/ µl |
| STRATAGENE™ MX3000P®            | 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.

#### R.2 ANALYTICAL 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.).

- 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.
- The specificity was improved through the selection of stringent reaction conditions.
- Genomic DNA isolated from bacteria potential interfering organisms with Chlamydia trachomatis were obtained from American Type Culture Collection (ATCC) and Vircell 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 |

### R.3 DIAGNOSTIC SPECIFICITY AND SENSITIVITY

#### R.3.1 Diagnostic Specificity:

Diagnostic specificity is the probability that the device gives a positive 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 35 C. trachomatis DNA negative samples (18 urine and 17 cervical swabs):

|                      |            |
|----------------------|------------|
| TRUE NEGATIVES       | 35         |
| FALSE POSITIVES      | 0          |
| TOTAL SAMPLES        | 35         |
| <b>SPECIFICITY %</b> | <b>100</b> |

On the basis of the results obtained **Diagnostic Specificity of the system has been calculated  $\geq 99\%$ .**

#### R.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 10 C. trachomatis DNA positive cervical swabs samples. The samples have been studied in duplicate in the same run and then it was been calculated the percentage (%) of positive samples.

|                      |            |
|----------------------|------------|
| TRUE POSITIVES       | 10         |
| FALSE NEGATIVES      | 0          |
| TOTAL SAMPLES        | 10         |
| <b>SENSITIVITY %</b> | <b>100</b> |

In addition, was tested the QCMD 2007 and the QCMD 2003 Chlamydia trachomatis (CTDNA07 and CTDNA03) EQA

Programme with 8 positive samples (4 urine and 4 swab) and 2 negative samples (1 urine and 1 swab) for each panel considered.

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

|                               |          |
|-------------------------------|----------|
| <b>Diagnostic Sensitivity</b> | 100 %    |
| <b>Diagnostic Specificity</b> | > 99.5 % |

### R.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%)  $\leq 10\%$ .**  
**Inter-Assay Coefficient Variation (CV%)  $\leq 10\%$ .**

### S. 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   |                            |   |                       |
|---|----------------------------|---|-----------------------|
|  | Product code               |  | Storage temperature   |
|  | In Vitro Diagnostic Device |  | See use instructions  |
|  | Lot number                 |  | Manufacturer          |
|  | Expiry date                |  | Number of tests       |
|  | CE conformity mark         |  | Date of manufacturing |

|   |
|---|
| <p>Produced by<br/>         Dia.Pro. Diagnostic Bioprobes Srl.<br/>         via Columella n°31 – Milano - Italy</p> |
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