High dynamic range detection of *Chlamydia trachomatis* growth by direct quantitative PCR of the infected cells

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**A B S T R A C T**

Chlamydiae are obligate intracellular bacteria developing in an intracytoplasmic niche, the inclusion. Chlamydia growth measurement by inclusion counting is a key task in the development of novel antichlamydial antibiotics and in vaccine studies. Most of the current counting methods rely on the immunofluorescent staining of the inclusions and either manual or automatic microscopy detection and enumeration. The manual method is highly labor intensive, while the automatic methods are either medium-throughput or require automatic microscopy. The sensitive and specific PCR technology could be an effective method for growth related chlamydial DNA detection; however the currently described PCR approaches have a major limitation, the requirement of purification of DNA or RNA from the infected cells. This limitation makes this approach unfeasible for high-throughput screenings. To overcome this, we developed a quantitative PCR (qPCR) method for the detection of *Chlamydia trachomatis* DNA directly from the infected HeLa cells. With our method we were able to detect the bacterial growth in a 4 log scale (multiplicity of infection (MOI): 64 to 0.0039), with high correlation between the biological and technical replicates. As a further proof of the method, we applied the direct qPCR for antibiotic minimum inhibitory concentration (MIC) measurements. The measured MICs of moxifloxacin, tetracycline, clarithromycin and compound PCC00213 were 0.031 μg/ml, 0.031 μg/ml, 0.0039 μg/ml and 6.2 μg/ml respectively, identical or close to the already published MIC values. Our direct qPCR method for chlamydial growth and antibiotic MIC determination is less time-consuming, more objective and more sensitive than the currently applied manual or automatic fluorescent microscopy-based methods.

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1. Introduction

*Chlamydia trachomatis* (*C. trachomatis*) ocular serovars A–C are responsible for acute and chronic inflammations of the conjunctiva and urogenital serovars D–K are involved in acute and chronic urogenital tract infections, while LGV serovars (L1–L3) cause lymphogranuloma venereum, a chlamydial sexually transmitted disease (STD) with systemic manifestations. The frequently chronic feature of these infections could lead to debilitating consequences such as ectopic pregnancy, infertility and blindness. *C. trachomatis* urogenital infections are the most frequent bacterial STDs in the world, with approximately 1.4 million infections yearly in the US (“CDC Sexually Transmitted Disease Surveillance, 2013: http://www.cdc.gov/std/stats13/std-trends-508.pdf,” n.d.). Approximately 10%–15% of untreated chlamydial infections lead to pelvic inflammatory disease (PID), and about 10%–15% of PID cases lead to infertility (Haggerty et al., 2010; Oakeshott et al., 2010).

Trachoma, the progressive fibrotic inflammation induced by *C. trachomatis* ocular serovars, is the leading cause of preventable blindness worldwide with 0.5 million people blind and 232 million people being at risk of acquiring the disease (“WHO | Trachoma Fact sheet No 382, http://www.who.int/mediacentre/factsheets/fs382/en/,” 2014). Azythromycin and doxycycline treatment of chlamydia infections is considered to be effective, however treatment failures reached 15–19% in certain studies (Golden et al., 2005; Katz et al., 1998), suggesting that novel antibiotics and/or antibiotics combinations should be evaluated. An effective vaccine could prevent the sequelae of chronic infections, however the effective vaccine targets and delivery modes have not yet been identified. Importantly, both the antibiotic and vaccine research require the accurate and preferentially high dynamic range measurement of the decrease of *C. trachomatis* growth at different time points and under various treatment regimens. Since the experimental parameters combine in an exponential manner, the readout method should be able to handle a large number of specimens in a high-throughput way. The highly sensitive and specific PCR method has been commonly used for the detection of *C. trachomatis* DNA in...
clipsial samples (Ouzounova-Raykova et al., 2015; Abou Tayoun et al., 2015; Dhawan et al., 2014; Watson et al., 2002). The qPCR method has also been used for the detection of the impact of different antibiotics on the growth of *C. trachomatis*, measuring the DNA or RNA concentration as a readout. This technology accurately characterized the MICs of ofloxacin, moxifloxacin, azithromycin, doxycycline on *C. trachomatis* (Peuchant et al., 2011a). In another study the doxycycline and azithromycin MICs of the Chlamydia-related bacterium *Waddlia chondrophila* have been characterized by qPCR (Goy and Greub, 2009).

The fundamental problem with these methods is that before the qPCR they require nucleic acid extraction from the infected cells, hence the analysis of more than one or two dozen samples is not feasible. To avoid column based DNA purification, we developed a method where we extract the DNA from the infected cells by two freeze–thaw cycles, and perform a direct qPCR of the lysed cells. This streamlined protocol needed significantly less hands on time than the traditional qPCR and proved to be highly sensitive. As a proof of principle, we were able to detect the *C. trachomatis* growth over a 4 log concentration range and accurately detect the *C. trachomatis* MICs of known and novel antibiotics.

2. Materials and methods

2.1. Chlamydia strain propagation

*C. trachomatis* serovar D reference strain (UW-3/CX, ATCC) was used in this study, and the chlamydia strain was propagated and partially purified as described previously (Sabet et al., 1984).

2.2. Culture of HeLa cells, chlamydia infection and DNA extraction

HeLa 229 cells (ATCC) were transferred into the wells of the 96-well plate (Sarstedt, Nümbrecht, Germany) at a density of 6 × 10⁴ cells/well in 100 μl of minimal essential medium (MEM) with Earle salts supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mmol/l L-glutamine, 1× non-essential amino acids, 8 mmol/l HEPES, 25 μg/ml gentamycin. The cells were incubated for 1 h at room temperature (RT) and then overnight at 37 °C, 5% CO₂ to obtain a 90% confluent cell layer. For *C. trachomatis* infection, the wells were washed twice with 200 μl/well of phosphate buffered saline (PBS), pH 7.4. HeLa cells were infected at various MOIs depending on the experiment. Inoculated cells in 100 μl 0.5% (w/v) glucose medium were centrifuged at 800 g for 60 min, RT. After infection, the cells were washed twice with PBS and culture medium containing 1 μg/ml cycloheximide and 2-fold dilutions of the respective antibiotics were added. The plates were incubated at 37 °C, 5% CO₂ for 48 h. After the 48 h infection, the cells were processed in 3 different ways: i) cell supernatants were used directly as a template for qPCR ii), the cells with the supernatants were subjected to 2 × freeze–thaw cycles with a quick freezing (−80 °C, 15 min) and a quick thawing on a plate shaker at RT. After the lysis, the cell lysates were thoroughly mixed including the edges of the wells using a multi-channel pipette. The mixed lysates were used as a template in the qPCR iii), the supernatants of the cells were removed and the cells were washed with PBS twice. After the second wash 100 μl Milli-Q (MQ) water (Millipore, Billerica, MA, USA) was added to each well and subjected to two freeze–thaw cycles and mixing as described above. The mixed lysates were used as a template in the qPCR. All cell culture reagents were purchased from SIGMA, St. Louis, MO, USA, unless otherwise indicated.

2.3. Inhibition of chlamydia growth with antibiotics

Moxifloxacin (Avelox; Bayer Pharma AG, Berlin, Germany), tetracycline hydrochloride (SIGMA, St. Louis, MO, USA) and clarithromycin (Klacid; Abbott, Chicago, IL, USA) were diluted in culture medium (see above). Concentration ranges of 0.25–0.002 μg/ml for moxifloxacin, tetracycline and clarithromycin with 2-fold dilutions were tested. The stock solution of antibacterial drug candidate PCC00213 (10 mg/ml) was prepared in dimethyl sulfoxide (DMSO) (SIGMA, St. Louis, MO, USA) and further diluted in culture medium; the applied concentration range was 50–0.391 μg/ml with 2-fold dilutions. After infection of HeLa cells with *C. trachomatis* at a MOI 16, the culture medium with 1 μg/ml cycloheximide was supplemented with the serial 2-fold dilutions of the respective antibiotics and was added to triplicate wells. The cell lysis/DNA extraction was performed according to the above described iii), protocol.

2.4. Direct quantitative PCR

qPCR was performed using the Bio-Rad CFX96 real-time system. Initially both the Maxima SYBR Green qPCR Master Mix (Thermo Scientific, Waltham, MA, USA) and the SsoFast EvaGreen qPCR Supermix (Bio-Rad, Hercules, CA, USA) master mixes were used, but after the performance comparison the SsoFast EvaGreen qPCR supermix were used in the further reactions. The qPCRs initially were performed with the *C. trachomatis* 16S RNA, ompA, ftsK, and pykF gene specific primer pairs. The primer sequences were the following: 16S tRNA: 5′-CACA AGCAGTTGACATGTTGT-3′, 5′-ACTAACGATAAGGGTCTGCTCTG-3′, ompA: 5′-TCGACGGAATTC TGTGGGAAGGTT-3′, 5′-TATCAGTTGTAG GTTGCCACACA-3′, ftsK: 5′-AATCTGGAAGCTTCATGACC-3′, 5′- ATACATCTGAAAGGGCTGT-3′, pykF: 5′-GTGGCAAAACGGTTTACGATGGA-3′, 5′-TGCAATACAGGATGCGCTTCAA-3′. After the sensitivity and specificity comparisons, the pykF primer pairs were chosen for the further reactions. The PCR mixture consisted of 5 μl SsoFast EvaGreen supermix, 1–1 μl forward and reverse primers (10 pmol each), 1 μl template, and 2 μl MQ water to 10 μl final volume. After the 10 min at 95 °C polymerase activation step, 40 PCR cycles of 20 s at 95 °C and 1 min at 64 °C were performed. The fluorescence intensity was measured at the end of the annealing-extension step. The specificity of amplification was confirmed by melting curve analysis. For each PCR, the cycle threshold (Ct) corresponding to the cycle where the amplification curve crossed the base line was determined. Student’s t-test was used to evaluate the statistical differences between the samples (3 biological replicates for each condition). For the statistical comparison both the Ct values and the relative DNA concentration values were used. For the relative DNA concentration calculation, the Ct data was normalized so that the average measured *C. trachomatis* DNA concentration at the highest applied antibiotic concentration was considered 1 and the relative DNA concentrations of the subsequent samples were compared to this value using the 2⁻⁰ΔΔCt formula.

2.5. DNA and RNA purification by column purification methods

To compare the detection sensitivity of the direct qPCR method and the traditional nucleic acid extraction protocols, HeLa cells were cultured in a 96 well plate, infected with *C. trachomatis* at MOI 64 and MOI 1 and incubated for 48 h as described above. After the 48 h infection, the cells were processed in 4 different ways: i) the cells with the supernatants were subjected to 2 × freeze–thaw cycles and mixing as described above ii) the supernatants of the cells were removed and the cells were washed with PBS twice, and 100 μl MQ water was added to the wells and subjected to two freeze–thaw cycles and mixing iii) the cellular DNA was extracted by a Quick-gDNA MiniPrep column purification kit (Zymo Research, Irvine, CA, USA) according to the manufacturer’s instructions, and eluted in 40 μl elution buffer iv; the cellular RNA was extracted by a Quick-RNA MiniPrep column purification kit (Zymo Research, Irvine, CA, USA) according to the manufacturer’s instructions, and eluted in 40 μl nuclease-free buffer. 10 μl total RNA was reverse transcribed by the qScript cDNA Synthesis Kit (Quanta Biosciences, Gaithersburg, MD, USA) according to the manufacturer’s instructions. Before the qPCR the cDNA product was diluted with 40 μl
MQ water. The ompA and the 16S rRNA qPCRs were performed with the primer pairs described above.

3. Results

3.1. Primer and master mix selection for direct qPCR

Four primer pairs targeting the ftsK, ompA, pykF genes and the 16S rDNA region of the C. trachomatis genome were selected for further evaluation. The template of the qPCR reactions were crude cell lysate from HeLa cells infected with C. trachomatis (MOI 4) in a 96 well plate, and lysed by two freeze–thaw cycles at 48 h post infection. The ideal primer pairs should have detected the C. trachomatis DNA with high sensitivity in a crude cell lysate template, but should not have bound to and amplified the host genomic DNA, and should not have formed primer–dimers. The four primer sequences were selected so that the annealing-temperatures were close to 60 °C, enabling us to use a two-step PCR. Amplification plots (not shown) and melting curve analysis (Fig. 1A–D) showed that all of the primers were able to detect the C. trachomatis DNA. In the uninfected host cell lysate samples, the melting curve analysis showed that the ompA and especially the ftsK and 16S rDNA primers amplified a 75–77 °C PCR product resembling primer–dimer formation, and a 85–89 °C PCR product indicating aspecific amplification in the absence of the C. trachomatis DNA template. This was especially noticeable for the 16S rDNA. On the other hand, the pykF primers showed absolutely no primer–dimer formation and aspecific amplification, indicating that the chance of misamplification will be low even when the chlamydial DNA concentration is much lower than the host’s. To further test the specificity, we used the pykF primer pairs with DNA from Escherichia coli, Enterococcus faecalis, Staphylococcus aureus and Staphylococcus epidermidis. No amplification was detected in these qPCRs. Finally, the pykF primer pairs were tested in silico for aspecific binding using the NCBI Primer-BLAST algorithm (Ye et al., 2012) on the RefSeq genome database. No hits besides the various C. trachomatis serovars were detected.

For the further qPCRs the pykF primer pairs were selected. The optimal annealing temperatures of the pykF primers were screened between 60 °C–72 °C by a gradient PCR.

Amplification plots and melting curve analyses (Fig. 2A–B) showed that the highest sensitivity was achieved when the annealing temperature was 64 °C. Finally, two qPCR master mixes containing either the Sso7d fusion DNA polymerase or a regular Taq DNA polymerase were compared for the optimal amplification of the non-purified C. trachomatis DNA template. Fig. 3 shows that the Sso7d fusion DNA polymerase produced higher relative fluorescent unit (RFU), and more importantly approximately 2 cycles lower Ct (about 4 fold higher sensitivity) than the Taq DNA polymerase. The Sso7d fusion DNA polymerase was selected for further qPCRs.

3.2. Dynamic range of the direct qPCR

To measure the dynamic range of the qPCR method, HeLa cells were infected with 1:2 serially diluted C. trachomatis ranging from MOI 64 to 0.0039. DNA was extracted from the infected cells by two freeze–thaw cycles at 48 h post infection, and 1 μl of the lysate was used directly in a qPCR reaction. The qPCR method was able to detect the C. trachomatis DNA quantitatively in all of the MOIs applied (Fig. 4). The Ct values showed a high correlation (R²: 0.995) with the theoretical Ct values through the whole 64–0.0039 (1/256) MOI range, indicating that the direct qPCR method has a dynamic range of approximately 16,400 fold (64/0.0039). It has to be noted that the C. trachomatis DNA could be detected when we used less than 0.0039 MOI, but the Ct values did not correlate well with the theoretical Ct values (data not shown); MOIs over 64 were not tested.

![Fig 1](image.png)

Fig 1. Primer selection for the direct qPCR. HeLa cells were infected with C. trachomatis, MOI 4. At 48 h post infection, DNA was extracted from the infected cells by two freeze–thaw cycles, mixed and 1 μl of the lysate was used directly in a qPCR reaction using a 60 °C annealing temperature and the SsoFast EvaGreen master mix (Bio-Rad). Melting temperature profiles are shown for the C. trachomatis specific primers ftsK (A), ompA (B), pykF (C) and 16S rDNA (D). The thin lines show the qPCR melting temperature profiles of C. trachomatis infected cells, the bold lines show the melting temperature profiles of the uninfected cells.
3.3. Comparison of *C. trachomatis* detection sensitivities using different nucleic acid extraction methods

In order to assess the detection sensitivity of the direct qPCR method and the traditional nucleic acid extraction protocols, we infected HeLa cells with *C. trachomatis* at MOI 64 and MOI 1 and incubated for 48 h. At the end of the incubation, the cells were processed in 4 different ways: i. the cells in their supernatants were subjected to 2× freeze–thaw cycles ii. the cells were washed with PBS twice, and MQ water was added to the wells before the two freeze–thaw cycles iii. the cellular DNA was extracted by a column purification method iv. the cellular RNA was extracted by a column purification method and reverse transcribed (Fig. 5). For both MOIs, the qPCR data showed that the lowest Ct-s (the highest sensitivities) could be measured for the 16S rRNA expression, followed by the direct lysis of the infected cells in MQ water, the column DNA purification method, the *ompA* gene expression and finally the direct lysis of the infected cells in their media. The similar Ct standard deviations indicated, that the reproducibilities of the different detection methods were comparable.

3.4. Measurement of the sensitivity and technical reproducibility of the direct qPCR in the presence of antibiotics

To assess the technical reproducibility of the direct qPCR, we infected HeLa cells with *C. trachomatis* (MOI 16) in the presence of 1:2 dilutions of (A) moxifloxacin and (B) tetracycline (Fig. 6 A–B). At 48 h post infection, infected cells were either left unwashed in the original medium or washed twice with PBS and finally 100 μl MQ water was measured on the cells. The DNA extraction was performed by two freeze–thaw cycles. Importantly, before the qPCR, the lysates (~100 μl) were mixed 5–6 times with a multichannel pipette (50 μl stroke volume) including the edges of the wells. qPCR resulted in approximately 1.5–2 lower Ct value (2.8–4 fold higher sensitivity) when the cells were washed and resuspended in MQ water before lysis compared with the lysis in the original medium. The further antibiotic growth inhibition measurements were performed with washed cells. To assess the reproducibility of the direct qPCR, 1 μl of the same lysate from one dilution series was measured three times (Fig. 6 C–D). The Ct values demonstrated excellent technical reproducibility in the case of both the moxifloxacin and...
tetracycline inhibition experiments. Pearson’s correlation coefficients among the three replicates ranged from 0.9921–0.9962 and 0.9980–0.9992 in the case of moxifloxacin and tetracycline inhibition experiments, respectively. For the tested 8 moxifloxacin and 8 tetracycline samples, the standard deviations never exceeded the 2% of the average of the 3 technical replicates.

3.5. C. trachomatis MIC value estimation of known and novel antibiotics by direct qPCR

The direct qPCR method was used to determine the MIC of known antichlamydial antibiotics moxifloxacin, tetracycline and clarithromycin and the novel antibiotic compound PCC00213. HeLa cells were infected with C. trachomatis (MOI 16) in the presence of 1:2 dilutions of moxifloxacin (Fig. 7A), tetracycline (Fig. 7B), clarithromycin (Fig. 7C) and compound PCC00213 (Fig. 7D) in 3 parallel wells. At 48 h post infection, infected cells were washed twice with PBS and finally 100 μl MQ water was measured on the cells. The DNA extraction was performed by two freeze–thaw cycles. After mixing the cell lysate, 1 μl of the lysate was used directly in a qPCR reaction. For MIC value estimation, the chlamydial DNA concentrations (Ct values) measured in the three parallel wells of a given antibiotic concentration were compared with the Ct values measured in the three parallel wells of the highest antibiotic concentration (we considered it as the inoculum) using Student’s t-test. The lowest antibiotic concentration, where the Ct values did not change significantly compared with the inoculum was considered the MIC value. The moxifloxacin MIC for C. trachomatis was measured previously as 0.03–0.05 μg/ml (Peuchant et al., 2011; Donati et al., 1999; Shima et al., 2011; Bogdanov et al., 2014). The qPCR showed that the last moxifloxacin concentration that did not change significantly compared with the inoculum was 0.031 μg/ml. The tetracycline MIC for C. trachomatis was characterized before as 0.03–0.15 μg/ml (Peuchant et al., 2011; Ikeda-Dantsuji et al., 2011; Welsh et al., 1992; Bogdanov et al., 2014). The qPCR showed that the last tetracycline concentration that did not change significantly compared with the inoculum was 0.031 μg/ml. The clarithromycin MIC for C. trachomatis was characterized before as 0.0078–0.032 μg/ml (Samra et al., 2001; Zheng et al., 2015). The qPCR showed that the last clarithromycin concentration that did not change significantly compared with the inoculum was 0.0309 μg/ml. The compound PCC00213 MIC for C. trachomatis serovar D was characterized before as 6.25 μg/ml (Bogdanov et al., 2014). The qPCR showed that the last PCC00213 concentration that did not change significantly compared with the inoculum was 6.25 μg/ml.

4. Discussion

Manual counting of chlamydial inclusions requires excessive hands-on time, technical skills, materials and equipments. Various alternative methods have been developed to circumvent manual counting but these methods are usually medium-throughput (Bogdanov et al., 2014), have a limited dynamic range (Southern et al., 2012; Osaka and Hefty, 2013; Osaka et al., 2012; Bogdanov et al., 2014), aspecific (Osaka and Hefty, 2013) or require expensive equipments, such as automated fluorescent microscope (Osaka et al., 2012). The limited dynamic range (~1 log) that is a feature of most of the current methods is especially a burden when the viable Chlamydia count, the so-called recoverable IFU, is measured from infected cells or tissues. In this case several dilutions should be prepared to determine the countable inclusion number, and each dilution counting should be performed in two–three parallels. As a practical alternative, we developed a highly sensitive, streamlined method for the detection of C. trachomatis growth, which only requires a widely available qPCR machine. To circumvent the bottleneck of the qPCR method, the DNA purification, we used lysates of the infected cells as a template. To achieve optimal qPCR performance using the unpurified DNA, we optimized the major parameters of the reaction. Interestingly, the most obvious choice, the 16S rDNA primers showed primer dimer formation and aspecific amplification, which might be due to the aspecific binding to human rDNA sequences and/or amplification of bacterial DNA remnant from the recombinant expression of the DNA polymerase. Since the qPCR uses crude cell lysate as a template, the inhibition of the DNA polymerase could be a problem. We tested qPCR master mixes with Taq polymerase and the Sso7d single stranded DNA binding protein fused DNA.
polymerase and concluded that the fusion DNA polymerase had an approximately 4 fold higher sensitivity. It has been described before, that the fusion makes the DNA polymerase highly efficient and inhibitor resistant, an ideal enzyme for direct qPCR (Wang et al., 2004). Nevertheless the qPCR may be performed with Taq polymerase as well, especially when the cells are washed before lysis. As a most easily available template, we tested cell culture supernatant, and also crude cell lysate. While the supernatant would be the simplest choice for direct qPCR, the sensitivity using this template was 10–48 fold less than that using crude cell lysate (data not shown). When we tested crude cell lysate we were able to follow the chlamydial growth between MOI 64–0.039 (1/256) with excellent correlation with the theoretical values, thus our method may be ideal for measuring the recoverable IFU of chlamydia. It should be noted that since the host cell number was relatively constant in our experiments, in order to make the protocol the simplest we did not note that since the host cell number was relatively constant in our experiments, in order to make the protocol the simplest we did not remove the culture medium, washing and resuspending in MQ water is advisable before the freeze thaw cycles. After mixing the cell lysate, 1 μl of the lysate was used directly in a qPCR reaction. Representative of two experiments. (B) To assess the reproducibility of the qPCR, the same templates from the infected and tetracycline (C) and moxifloxacin (D) treated cells were measured three times using lysates from washed and MQ suspended cells. The Pearson correlation coefficients calculated among the three technical replicates are also shown.

![Graphs A, B, C, D](image)

**Fig 6.** Measurement of the sensitivity and technical reproducibility of the direct qPCR in antibiotic growth inhibition experiments. HeLa cells were infected with C. trachomatis, MOI 16 in the presence of various concentrations of (A) tetracycline (0.25 μg/ml–0.002 μg/ml) and (B) moxifloxacin (0.25 μg/ml–0.002 μg/ml). At 48 h post infection, infected cells were either washed twice with PBS and finally 100 μl MQ water was measured on the cells or the cells were left unwashed. The DNA extraction was performed by two freeze-thaw cycles. After mixing the cell lysate, 1 μl of the lysate was used directly in a qPCR reaction. Representative of two experiments. (B) To assess the reproducibility of the qPCR, the same templates from the infected and tetracycline (C) and moxifloxacin (D) treated cells were measured three times using lysates from washed and MQ suspended cells. The Pearson correlation coefficients calculated among the three technical replicates are also shown.

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Fig 7. Measurement of MICs of known and novel antimicrobial compounds. HeLa cells were infected with C. trachomatis, MOI 16 in the presence of various concentrations of moxifloxacin (A), tetracycline (B), clarithromycin (C) and compound PCC00213 (D). Each infection at a particular antibiotic concentration was performed in parallel wells. At 48 h post infection, infected cells were washed twice with PBS and finally 100 μl MQ water was measured on the cells. The DNA extraction was performed by two freeze–thaw cycles. After mixing the cell lysate, 1 μl of the lysate was used directly in a qPCR reaction. Data are the average – Ct values and the standard deviations. Inserts show linear qPCR data that was normalized so that the average measured C. trachomatis DNA concentration at the highest applied antibiotic concentration was considered 1 and the relative DNA concentrations of the subsequent samples were compared to this value using the 2^−ΔΔct formula. Data are means ± standard deviations of the parallel samples. *: the relative DNA concentration values or the Ct values are significantly different (p < 0.05) from the values measured in the presence of the highest concentration of antibiotics using Student’s t-test.

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We hope that our method will help to democratize chlamydial growth monitoring and encourage the research of novel antichlamydial compounds and mechanisms. Also, the direct qPCR method can be applied for the growth screening of other intracellular pathogens.


