



HAL
open science

DNA stability of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine

Rémi Le Guern, Brigitte Miaux, Patricia Pischedda, Stéphanie Herwegh, René Courcol

► **To cite this version:**

Rémi Le Guern, Brigitte Miaux, Patricia Pischedda, Stéphanie Herwegh, René Courcol. DNA stability of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine. *Diagnostic Microbiology and Infectious Disease*, 2016, 85 (3), pp.313-314. 10.1016/j.diagmicrobio.2016.04.005 . hal-02566184

HAL Id: hal-02566184

<https://hal.science/hal-02566184v1>

Submitted on 7 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

TITLE: DNA stability of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine

Running TITLE: Bacterial DNA stability in urine

AUTHORS: Rémi Le Guern^{1,2}, Brigitte Miaux², Patricia Pischedda², Stéphanie Herwegh², René Courcol^{1,2}

INSTITUTIONS:

¹ Université de Lille, UFR Médecine, F-59000 Lille

² CHU Lille, Institut de Microbiologie, F-59000 Lille

Word counts: Abstract = 50 words / Body = 1068 words

CORRESPONDING AUTHOR: Rémi Le Guern

CHU Lille, Institut de Microbiologie, F-59000 Lille

e-mail : remi.leguern@gmail.com

Tel : 33-3-20-44-54-80

ABSTRACT

We evaluated the DNA stability of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in 55 urine samples. Quantification cycle values (Cq) values were highly similar after 3 to 14 days at room temperature (+0.002, p = 0.99). Consequently, it does not seem necessary to transfer urine specimens into a transport medium in less than 24 hours as recommended by manufacturers.

KEYWORDS

DNA stability; sexually transmitted infections; Cobas 4800 CT/NG; *Chlamydia trachomatis*; *Neisseria gonorrhoeae*

Bacterial sexually transmitted infections (STIs) are mainly caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Nucleic acid amplification techniques (NAATs) have recently been recommended by the Centers for Disease Control and Prevention to diagnose these STIs (1).

NAATs are often more sensitive than bacterial cultures when used to diagnose STIs, especially when the delay between sampling and arrival at the laboratory is substantial (2). Indeed, to cultivate *C. trachomatis* or *N. gonorrhoeae*, their viability must be maintained during transport, which is not an issue with NAATs. NAATs amplify a sequence of nucleic acids from a target microorganism, even if it is non-cultivable or dead.

For NAATs, the maximum length of time recommended between sampling and analysis depends on the type of sample (urogenital swabs or urine), the manufacturer, and the utilization of a transport medium. Urogenital swabs are usually supplied with a transport medium and can be conserved for extended periods: 60 days for the Aptima Combo 2 assay (Hologic, San Diego, CA); 45 days for the Xpert CT/NG assay (Cepheid, Sunnyvale, CA); and 12 months for the Cobas 4800 CT/NG (Roche Diagnostics, Indianapolis, IN). However, manufacturer recommendations are more strict for urine samples without a transport medium: 24 hours for the Aptima Combo 2; 24 hours at room temperature or 8 days at 4°C for the Xpert CT/NG; and 24 hours for the Cobas 4800 CT/NG. Once the urine sample has been transferred into a transport medium, the specimen can be stored for 30 days for the Aptima Combo 2, 45 days for the Xpert CT/NG, and 365 days for the Cobas 4800 CT/NG (1).

Depending on the organization of a given laboratory, it is not always possible to immediately transfer urine samples received during weekends into an adequate transport medium. Specifically, a urine sample received on Friday night may be transferred into a transport medium on Monday morning, more than 48 hours after arrival. If the target DNA is degraded, it could lead to false negative results. The aim of this study was to evaluate the DNA stability of *C. trachomatis* and *N. gonorrhoeae* in urine samples without a transport medium using the Cobas 4800 CT/NG.

Urine samples for which *C. trachomatis* or *N. gonorrhoeae* DNA was detected using the Cobas 4800 CT/NG were included from January 2015 to April 2015. Samples received in the laboratory already in a transport medium were excluded. Upon arrival, the sample was separated into two aliquots: a first portion was transferred into a Cobas transport medium in less than 24 hours according to the manufacturer's recommendations; a second portion was conserved without transport medium at room temperature for at least 3 days. Qualitative results for the detection of *C. trachomatis* or *N. gonorrhoeae* DNA and quantification cycle values (Cq) of the two aliquots were compared. SPSS 23.0 (IBM Corp, Armonk, NY) was employed for statistical analysis. A Student t-test was utilized to compare paired samples.

Fifty-five urine samples were assessed in this study: *C. trachomatis* DNA was detected in 49 samples (89%) and *N. gonorrhoeae* DNA in 7 samples (13%). One specimen was positive for both *C. trachomatis* and *N. gonorrhoeae*. The median length of conservation without a transport medium at room temperature before analysis was 6 days (minimum 3 days, maximum 15 days).

All positive samples conserved in the Cobas transport medium were also positive in the group without transport medium (55/55). The mean of Cq values was 31.51 for both groups. It corresponds to a mean difference of 0.002 ($p = 0.99$), with a 95% confidence interval of [-0.42; 0.42]. Cq value differences between these two groups are shown in the Bland-Altman plot (Figure 1). Most of the Cq

differences were between -3 Cq and +3 Cq (55/57, 96.5%). Overall, this indicates that *C. trachomatis* and *N. gonorrhoeae* DNA appear stable in urine, even without the utilization of a transport medium, at room temperature for at least 3 days.

The stability of *C. trachomatis* DNA was first evaluated using conventional PCR by Morr  et al. (3). They reported a decrease in sensitivity after 3 to 4 days at room temperature as evaluated by a weaker amplicon signal following gel electrophoresis. The explanation provided by the authors was the presence in urine samples of DNase, which is more active at room temperature than at 4 C. In contrast, another study reported robust DNA stability of *C. trachomatis* after delays exceeding 4 days (4).

More recently, the stability of *C. trachomatis* DNA was examined using real-time PCR (5). Clinical and spiked samples were assessed for up to 2 years using different temperature and medium conditions, including urine stored at room temperature without transport medium. *C. trachomatis* DNA was still detected in all clinical samples and spiked media tested even after 2 years. Interestingly, it was noticed that there was a significant decrease in the number of cycles needed to detect *C. trachomatis* DNA in urine samples without transport medium within the first month of storage. These lower Cq values persisted even after 2 years of storage at room temperature or 4 C. On the other hand, long-term freezing for 2 years was associated with an increase of Cq values, implying that degradation of *C. trachomatis* DNA may have occurred. Overall, the conclusions were that storage conditions and duration did not affect *C. trachomatis* detection by PCR in a negative way, except for frozen urine samples (-20 C or -80 C) conserved for more than 2 years.

The DNA stability of *Trichomonas vaginalis*, a sexually transmitted parasite, was also assessed in urine samples (6). After 3 to 7 days at 4 C, *T. vaginalis* DNA was still detected in 95% (19/20) of the initially positive samples (6). However, after 3 to 7 days at room temperature, *T. vaginalis* DNA was only detected in 70% (14/20) of the initially positive samples. The authors found a Cq value difference of +3.6 after 7 days at 4 C and +4.4 after 7 days at room temperature. Another study described false negatives with *T. vaginalis* PCR after only 2 hours at room temperature (7). The DNA of *T. vaginalis* could be less stable in urine than the DNA of bacteria.

Manufacturers recommend transferring urine samples in transport medium in less than 24 hours to detect *C. trachomatis* or *N. gonorrhoeae* using NAATs. However, it seems that urine samples stored at room temperature for at least 3 days could also be acceptable. Using the Cobas 4800 CT/NG, it appears that it is not necessary to transfer a urine specimen into the transport medium immediately, which can ease the workload of a laboratory during weekends. It should be noted that these results cannot be generalized for other pathogens.

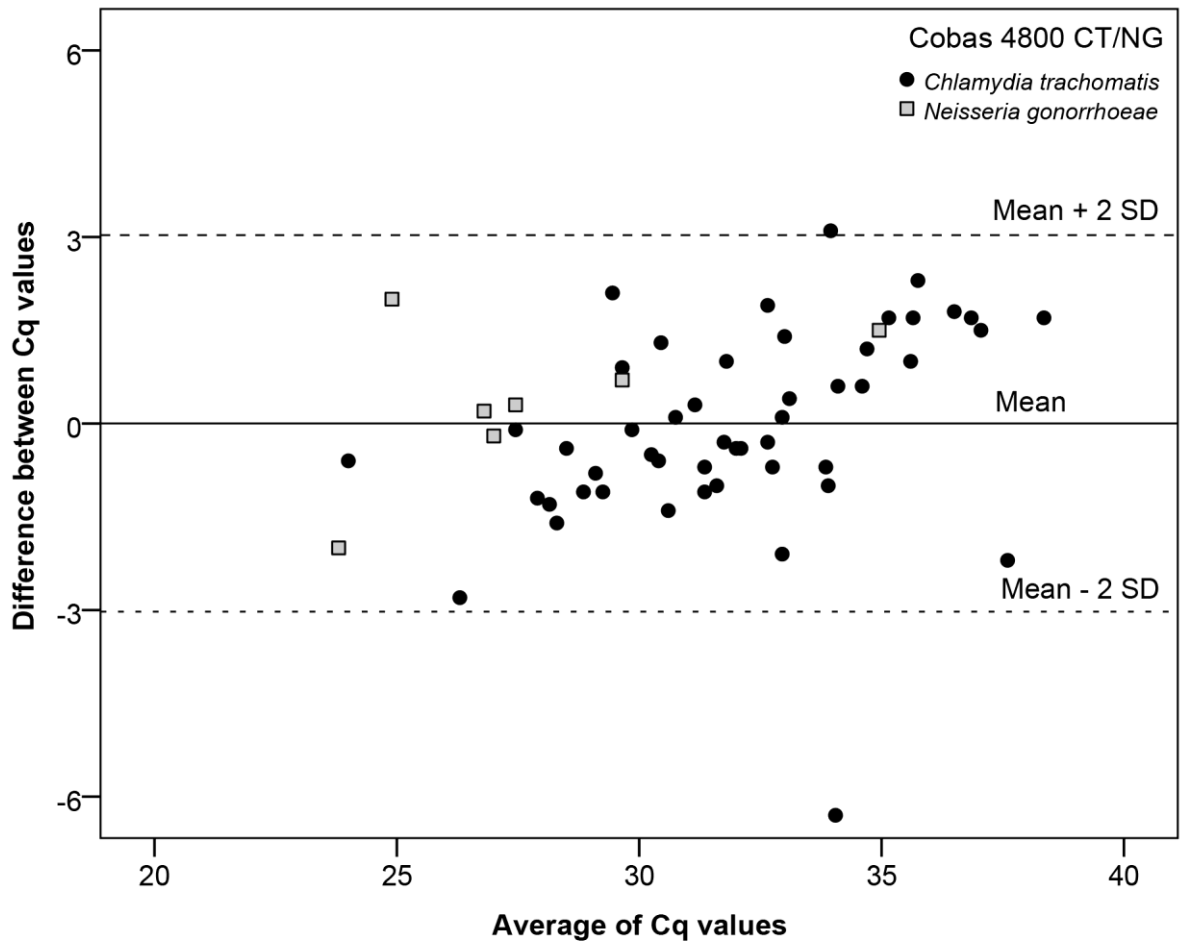


Figure 1. Bland-Altman plot: Differences between Cq values after at least 3 days in urine at room temperature without a transport medium.

Cq: Quantification cycle values

SD: Standard Deviation

BIBLIOGRAPHY

1. **Centers for Disease C, Prevention.** 2014. Recommendations for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*--2014. MMWR Recomm Rep **63**:1-19.
2. **Johnson RE, Newhall WJ, Papp JR, Knapp JS, Black CM, Gift TL, Steece R, Markowitz LE, Devine OJ, Walsh CM, Wang S, Gunter DC, Irwin KL, DeLisle S, Berman SM.** 2002. Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections--2002. MMWR Recomm Rep **51**:1-38; quiz CE31-34.
3. **Morre SA, van Valkengoed IG, de Jong A, Boeke AJ, van Eijk JT, Meijer CJ, van den Brule AJ.** 1999. Mailed, home-obtained urine specimens: a reliable screening approach for detecting asymptomatic *Chlamydia trachomatis* infections. J Clin Microbiol **37**:976-980.
4. **Skidmore S, Horner P, Herring A, Sell J, Paul I, Thomas J, Caul EO, Egger M, McCarthy A, Sanford E, Salisbury C, Macleod J, Sterne JA, Low N, Chlamydia Screening Studies Project G.** 2006. Vulvovaginal-swab or first-catch urine specimen to detect *Chlamydia trachomatis* in women in a community setting? J Clin Microbiol **44**:4389-4394.
5. **van Dommelen L, Wolffs PF, van Tiel FH, Dukers N, Herengreen SB, Bruggeman CA, Hoebe CJ.** 2013. Influence of temperature, medium, and storage duration on *Chlamydia trachomatis* DNA detection by PCR. J Clin Microbiol **51**:990-992.
6. **Ingersoll J, Bythwood T, Abdul-Ali D, Wingood GM, Diclemente RJ, Caliendo AM.** 2008. Stability of *Trichomonas vaginalis* DNA in urine specimens. J Clin Microbiol **46**:1628-1630.
7. **Shafir SC, Sorvillo FJ.** 2006. Viability of *Trichomonas vaginalis* in urine: epidemiologic and clinical implications. J Clin Microbiol **44**:3787-3789.