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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.

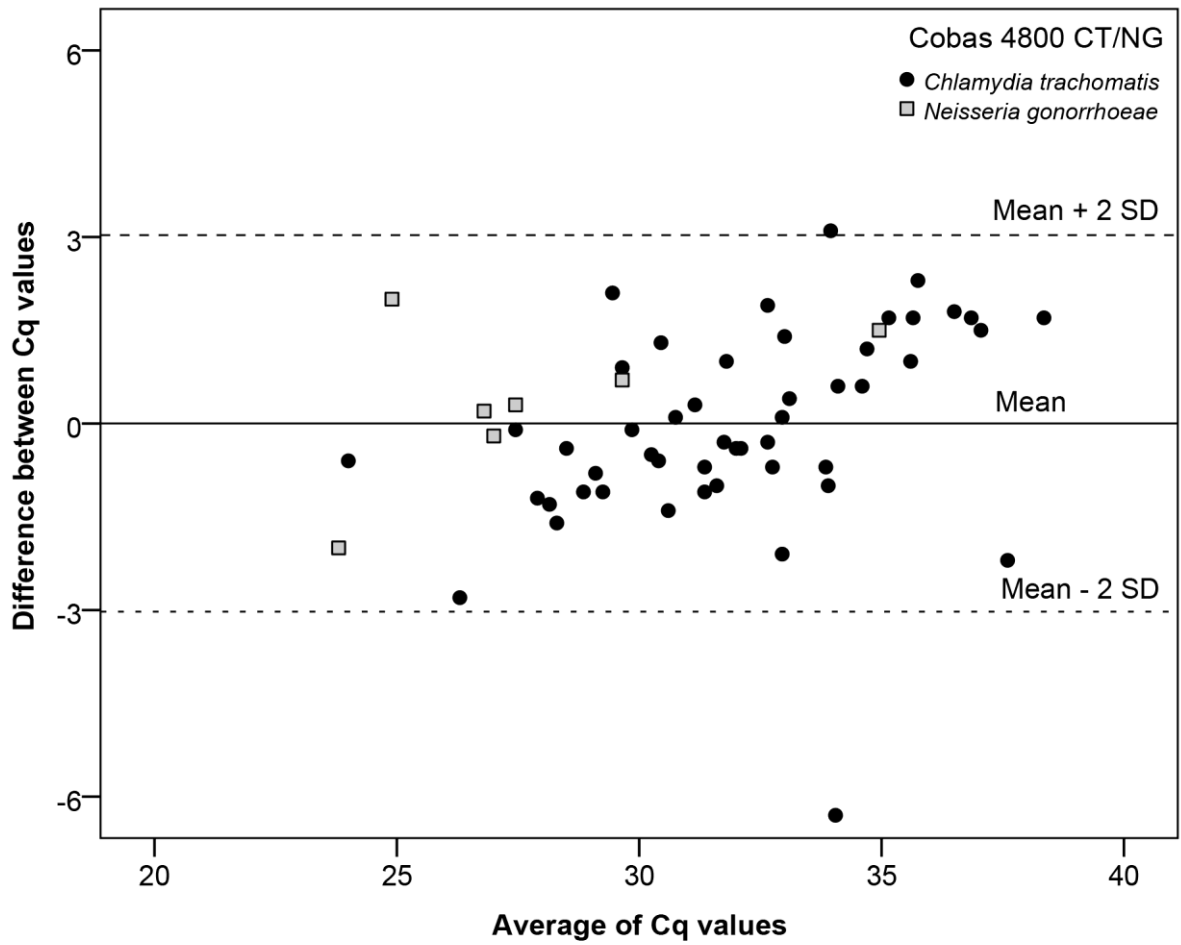


Figure 1. Bland-Altman plot: Differences between Cq values after at least 3 days in urine at room temperature without a transport medium.

Cq: Quantification cycle values

SD: Standard Deviation

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