Chlamydia trachomatis and *Neisseria gonorrhoeae* DNA stability in preserved first-void urine

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BACKGROUND & OBJECTIVES

The aim of this study was to evaluate the performance of UCM (Urine Conservation Medium, Novosanis, BE) for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) DNA preservation at room temperature (RT).

METHODS

To compare the stability of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) DNA in unpreserved and UCM-preserved samples, five CT and NG negative urine samples were spiked with Amplirun® *Chlamydia trachomatis* DNA control and Amplirun® *Neisseria gonorrhoeae* DNA control to obtain 99.0 CT and 75.5 NG DNA copies/µL in the final aliquot. For UCM-preserved samples, UCM was spiked, and urine was added in a 2:1 (urine:UCM) ratio to mimic real-life use of Colli-Pee® containing UCM.

A total of 30 aliquots were analyzed using the Abbott Real-Time CT/NG assay after storage at RT for 1, 8 and 15 days (Figure 1). Mixed-effect regression analysis was used to estimate the effect of UCM on CT and NG Ct values at each timepoint. Urine samples were introduced as random effects.

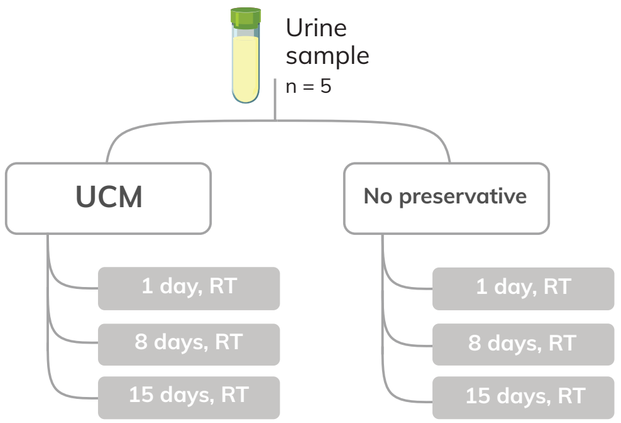

Figure 1: Visual representation of the experimental design.

RESULTS

After 15 days of storage, all UCM-preserved samples were still positive for CT and NG, while only one and three unpreserved sample remained positive for CT and NG respectively. Over time, Ct-values ranged from an average of 38.59 to 39.40 (CT), and 35.24 to 38.17 (NG) for unpreserved samples, and from 32.56 to 34.23 (CT), and 28.32 to 30.81 (NG) for preserved samples. Ct values where samples were negative for CT and/or NG were defined as the cut-off values (cut-off 8d: CT = 39.66, NG = 39.93; cut-off 15d: CT = 39.56, NG = 39.92). At each timepoint, Ct-values for both CT and NG were significantly lower for preserved compared to unpreserved samples (Figure 2 & 3).

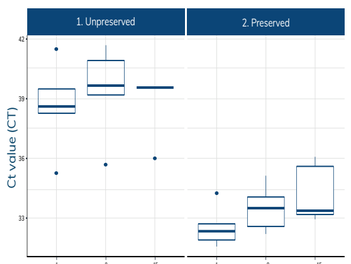

Figure 2: Visual representation of the raw data i.e., Ct values of CT for each preservation condition and time point.

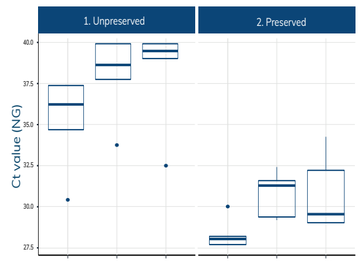

Figure 3: Visual representation of the raw data i.e., Ct values of NG for each preservation condition and time point.

Effect of preservation condition

Table 1 shows the effect of the preservation (i.e. unpreserved vs UCM-preserved) on Ct values after 1, 8 and 15 days of storage.

In addition to the visualization in Figure 2 & 3, these statistically significant results provide evidence that UCM is able to preserve CT DNA and NG DNA in urine. Already after 1 day of storage, the preservation of both CT and NG in the samples with UCM was significantly better compared to the unpreserved samples (i.e. >6 cycle numbers). This same trend was visible after 8 days and even after 15 days, were the preservation of CT and NG in samples with UCM was still significantly better (i.e. 4-7 cycle numbers). These results were obtained after spiking with approximately 100 copies/µL of both CT DNA and NG DNA at day 0 in all preservation conditions, namely unpreserved samples and samples preserved with UCM. All effects are statistically significant with p<0.01.

Table 1: Regression analysis to assess the effect of the preservation condition. Mixed effect linear models were used to account for repeated measurements from one sample. Cycle threshold for CT and NG was used as the outcome, and buffer condition was used as the independent variable with no preservative as a reference. The 95% confidence interval is shown between brackets, followed by the p-value. * represents a significant difference from the reference i.e., $p < 0.05$.

Outcome	Unpreserved	Preserved
CT - Ct 1d	Reference i.e., 38.59	-6.03 [-8.23, -3.84] ; p = 0.000655*
CT - Ct 8d	Reference i.e., 39.40	-5.91 [-7.88, -3.94] ; p = 0.00417*
CT - Ct 15d	Reference i.e., 38.84	-4.60 [-5.98, -3.23] ; p = 0.00281*
NG - Ct 1d	Reference i.e., 35.24	-6.91 [-9.59, -4.24] ; p = 0.000964*
NG - Ct 8d	Reference i.e., 37.98	-7.20 [-9.79, -4.62] ; p = 0.0000600*
NG - Ct 15d	Reference i.e., 38.17	-7.36 [-10.03, -4.68] ; p = 0.00571*

Effect of time of storage

Table 2 shows the effect of time of storage on preservation for both preservation conditions, namely unpreserved and preserved (UCM) samples. Over time, the preservation of NG was lower in both unpreserved and preserved samples. No significant effects were observed for CT over time in both unpreserved and preserved samples.

Table 2: Regression analysis to assess the effect of time. Mixed effect linear models were used to account for repeated measurements from one sample. Cycle threshold for CT and NG was used as the outcome, and time point was used as the independent variable with time point 1 as a reference. The 95% confidence interval is shown between brackets, followed by the p-value. * represents a significant difference from the reference i.e., $p < 0.05$.

Outcome	t = 1 day	t = 8 days	t = 15 days
CT - Ct unpreserved	Reference i.e., 38.59	0.81 [-0.16, 1.78]; p = 0.139	0.25 [-0.72, 1.21]; p = 0.631
CT - Ct preserved	Reference i.e., 32.56	0.93 [-0.62, 2.48]; p = 0.260	1.67 [0.12, 3.22]; p = 0.0558
NG - Ct unpreserved	Reference i.e., 35.24	2.74 [1.50, 3.98]; p = 0.00249*	2.93 [1.69, 4.17]; p = 0.00167*
NG - Ct preserved	Reference i.e., 28.32	2.45 [0.44, 4.46]; p = 0.0437*	2.49 [0.48, 4.50]; p = 0.0412*

CONCLUSION

UCM-preserved urine sampling enables preservation of CT and NG DNA for 15 days of storage at RT. The use of Colli-Pee®, whereby the collector tube is prefilled with UCM, allows for immediate mixing of urine and preservative. This offers opportunities for home-based testing where sample quality is maintained during storage and sample shipment to the laboratory by regular postal mail.

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