

# THE EFFECT OF STORAGE AT ROOM TEMPERATURE AND DELAYED TESTING ON Cq VALUES OF FOUR STI PCRS



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## Introduction

Home based self-sampling to test for sexually transmitted infections (STI) is becoming increasingly popular. Samples are usually sent via regular postal services and are not refrigerated during transport. Subsequently, samples are tested for STI with quantitative polymerase chain reaction (qPCR). To test the effect of storage at room temperature, we compared PCR outcomes for STI testing upon direct and delayed testing for a range of materials.

## Material and Methods

**Samples:** All urines and M-swabs (Copan, Italy) received between August and December 2016 for STI diagnostics were tested on day 0 and if positive for one of the 4 STI on day 7-9 after being stored at room temperature (Table 1).

Extractions were performed by VERSANT kPCR SP (Siemens) using 250 µl sample. M-swabs and urine were preserved as received; urines were transferred into VERSANT urine transport medium prior to analysis.

**STI testing:** We tested for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) on the VERSANT kPCR platform (Siemens, Germany) and for *Mycoplasma genitalium* (MG) and *Trichomonas vaginalis* (TV) using a laboratory-designed qPCR.

	CT	NG	MG	TV	TOTAL	
vaginal	32	12	28	10	82	47%
urine	28	12	19	-	59	34%
anal	6	8	7	-	21	12%
other	2	8	1	-	11	6%
<b>TOTAL</b>	<b>68</b>	<b>40</b>	<b>55</b>	<b>10</b>	<b>173</b>	
	39%	23%	32%	6%		

Table 1: qPCR test and material types that were positive.

**Statistical analysis:** We calculated the difference in Cq value at day 0 and after one week for each sample. These differences were averaged per target, per sample type, and per combination of target and sample type. A paired two-sided t-test with multiple hypothesis correction was performed to test for statistical significance.

## Results

We obtained 156 samples (67 vaginal, 58 urine, 18 anal, 13 other) from 154 patients. CT, NG, MG and TV were detected in 44%, 27%, 37% and 7% of samples, respectively. 24 (16%) of the patients were positive for two or three STI. Six samples were negative after one week (excluded from further analyses):

- 1 TV: initial Cq 27
- 1 CT: initial Cq 35
- 2 NG: initial Cq 33 and 38
- 2 MG: initial Cq 27 and 38

	CT	NG	MG	TV	MEAN	
vaginal	24.7 -0.1	26.5 -0.5	30.9 +0.8	23.1 +4.1	26.9 +0.7	p<0.001
urine	25.8 -1.0	21.8 -0.1	30.3 +0.0	-	26.4 -0.5	p<0.01
anal	27.2 -1.2	26.7 -0.6	33.5 +0.1	-	29.1 -0.5	p<0.05
other	30.1 -0.1	30.3 -1.1	26.3 -0.1	-	29.9 -0.8	
<b>MEAN</b>	<b>25.6</b> -0.6	<b>25.9</b> -0.5	<b>31.0</b> +0.4	<b>23.1</b> +4.1	<b>27.2</b> +0.0	

Table 2: Cq value at day 0, and changes after one week.

In Table 2 it can be seen that MG has a substantially higher average Cq value than the other three tests, both on day 0 and after one week.

The Cq values of TV increased significantly, with an average increase of Cq of 4.1 (p=0.0008), based on 10 samples. Before multiple hypothesis correction, a small decrease in Cq value was observed with the passage of one week for the aggregate CT and NG results.

## Conclusions

➡ CT, MG and NG testing after storing M-Swab or urine samples at room temperature for one week may be acceptable - although for each target one or two samples were negative when retesting.

➡ Cq values for TV were significantly weaker after room-temperature storage, suggesting that false negative results might occur if the testing delay is long. Further studies on the effects of delayed testing for TV are needed.

