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Inhibition of PCR in Genital and Urine Specimens Submitted for *Chlamydia trachomatis* Testing

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We determined the frequency of PCR inhibition in genital and urine specimens submitted for *Chlamydia trachomatis* testing using the internal control DNA provided with the COBAS AMPLICOR *C. trachomatis* test and assessed methods to remove it. Inhibition occurred in 65 of 906 (7%) cervical swabs, 23 of 51 (45%) urethral swabs, and 2 of 175 (1.1%) urine samples. Overall, inhibition was eliminated in processed specimens after storage at 4°C in 77 of 90 specimens (86%), freezing at −70°C in 59 of 82 specimens (72%), storage at 4°C followed by either 1:100 dilution in 37 of 43 specimens (86%) or 1:10 dilution in 42 of 47 specimens (89%), and phenol-chloroform extraction in 79 of 80 specimens (99%). No positive specimens were missed due to inhibition. We conclude that PCR inhibition is rare with urine specimens and infrequent with endocervical swabs but occurs frequently with urethral swabs. The frequency of PCR inhibition may be significantly reduced by methods which can be easily incorporated into the processing of specimens.

The use of nucleic acid amplification methods such as PCR has significantly improved our ability to diagnose genital *Chlamydia trachomatis* infections (3). These methods also allow for the use of noninvasive urine specimens for testing which are more acceptable to patients (3, 5, 10, 11, 15). The introduction of commercially available automated DNA amplification assays has allowed more laboratories to introduce these technologies for routine testing of specimens. However, there is some concern that certain substances in clinical specimens may inhibit these assays. In most studies, the frequency of inhibition has been determined by analyzing specimens which were negative by PCR but positive by one of the other methods being used in the comparison, such as culture (1, 2, 9, 10). Few studies have attempted to determine the inhibition rate for all specimens being tested (4, 14, 16). However, various methods of specimen pretreatment have been used in an attempt to neutralize inhibitors in the specimen with variable success (1, 2, 8–11, 16, 17).

The COBAS AMPLICOR *C. trachomatis* test (Roche Diagnostic Systems, Inc., Branchburg, N.J.) is a commercially available automated assay. Internal control (IC) DNA is provided and is added to the PCR Master Mix. This IC DNA contains primer-binding regions identical to those of the *C. trachomatis* target sequence but has unique internal-detection probe-binding regions. This allows simultaneous amplification of both *C. trachomatis* target DNA, when it is present in the clinical specimen, and IC target DNA, followed by selective detection of either amplicon. The failure to detect the IC target DNA after amplification indicates that inhibition of PCR has occurred (14). The objectives of this study were to determine the frequency of PCR inhibition in genital and urine specimens and to compare the effectiveness of various methods in removing inhibition.

Cervical swabs were obtained from women and urethral swabs from men receiving care at the Ottawa General Hospital. First-catch urine specimens (FCU) were obtained from asymptomatic street youth as part of an outreach study. Specimens were transported to the clinical microbiology laboratory at ambient temperature. FCU specimens were stored at 4°C, and the transport media (STM) from the swab specimens were kept at room temperature until testing. All specimens were processed and tested within 4 days of specimen collection as recommended by the manufacturer.

Specimens were batched and processed two to three times weekly by using the COBAS AMPLICOR *C. trachomatis* test reagents according to the manufacturer's instructions. Testing was performed with the COBAS AMPLICOR instrument on the same day on which specimen processing occurred. The presence of visible blood in the swab specimens was noted and recorded. Amplification followed by detection of both *C. trachomatis* and IC DNA was carried out on all specimens. The failure to detect the IC target DNA in *C. trachomatis*-negative specimens after amplification indicated that PCR inhibition had occurred (14).

For these inhibited specimens, the processed specimen was divided into 3 aliquots and treated as described below prior to retesting. One aliquot was stored at 4°C, another was stored at −70°C, and the third was phenol-chloroform extracted and ethanol precipitated. The aliquot stored at 4°C was retested undiluted and after dilution with specimen diluent. A 1:100 dilution was used for the first half of the study and a 1:10 dilution for the second half. IC DNA was added for all retesting as described above. As retesting of specimens demonstrating PCR inhibition were batched, the numbers of days during which the processed specimens were stored at 4°C prior to retesting varied from 1 to 7 days. For the last 2 months of the study, specimens demonstrating PCR inhibition were not subjected to phenol-chloroform extraction or freezing at −70°C. Groups were compared by χ^2 analysis with Yates' correction or by Fisher's exact test, with a P value of ≤ 0.05 being considered statistically significant.

C. trachomatis PCR was positive in 17 of 906 (1.9%) cervical swabs, 4 of 51 (7.8%) urethral swabs, 8 of 101 (8%) male FCU, and 4 of 74 (5.4%) female FCU. Inhibition of PCR was present in 65 of 906 (7.2%) cervical swabs, 23 of 51 (45.1%) urethral swabs, 2 of 74 (2.7%) female FCU, and none of the male FCU. Although not statistically significant, the inhibition rate for

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TABLE 1. Ability of treatment methods in eliminating PCR inhibition

Treatment procedure	No. of IC-positive specimens after treatment/no. treated (%)		
	Urethral swabs	Cervical swabs	Both types of swabs
Phenol-chloroform extraction	20/20 (100)[b]	57/58 (98.3)[a]	77/78 (98.7)[a]
Storage at −70°C	9/20 (45.0)[a]	48/60 (80.0)[b]	57/80 (71.2)[a]
Storage at 4°C	19/23 (82.6)	56/65 (86.2)	75/88 (85.2)
Storage at 4°C			
1:10 dilution	15/18 (83.3)[b]	27/29 (93.1)[b]	42/47 (89.4)[b]
1:100 dilution	4/5 (80.0)[b]	32/36 (88.9)[b]	36/41 (87.3)[b]

[a] $P < 0.05$, compared to storage at 4°C.
[b] $P > 0.05$, compared to storage at 4°C.

visibly bloody cervical specimens was higher than that for nonbloody specimens (3 of 20 [15%] versus 62 of 886 [7%]; $P = 0.18$). Cervical specimens received with the swab left in the STM had a significantly higher rate of PCR inhibition than specimens without the swab (5 of 27 [18.5%] versus 60 of 879 [6.8%]; $P = 0.03$). None of the urethral specimens was received with the swab left in the STM. The elapsed time interval (range, 0 to 4 days) from when specimens were received in the laboratory to initial processing and testing did not affect the inhibition rate (data not shown).

The ability of each treatment method to eliminate inhibition in the swab specimens is summarized in Table 1. Overall, phenol-chloroform extraction was the most-effective method in eliminating PCR inhibition, whereas freezing at −70°C was the least effective, especially for urethral swabs. Storage at 4°C and retesting without dilution were as effective as storage at 4°C with dilution (at both 1:10 and 1:100); these methods eliminated PCR inhibition in 80 to 93% of specimens. PCR inhibition in the two female FCU specimens was eliminated with phenol-chloroform extraction, storage at 4°C, and freezing at −70°C. Dilution to 1:100 eliminated inhibition in only one of the two FCU specimens. None of the specimens (swabs or urine) initially demonstrating PCR inhibition were positive for *C. trachomatis* upon retesting after any of the treatment methods.

The length of time during which processed specimens were stored at 4°C before retesting (with or without dilution) did not appear to affect the ability of these treatment methods in eliminating PCR inhibition. PCR inhibition was removed in 37 of 45 (82%) genital specimens which were retested undiluted after being stored for ≤3 days at 4°C compared to 38 of 43 (88%) genital specimens stored for >3 days ($P = 0.61$). Similar results were seen when the specimen was diluted either 1:10 or 1:100 (40 of 45 [89%] versus 38 of 43 [88%]; $P = 0.8$). However, there were only 15 specimens that were stored for ≤2 days at 4°C.

C. trachomatis PCR results were positive for 33 specimens, of which 28 were available for further analysis. These were all diluted 1:100 with specimen diluent and were retested for *C. trachomatis* DNA by PCR to determine the effect of dilution. All 18 genital swab specimens, 3 of 6 male FCU, and 3 of 4 female FCU were PCR positive for *C. trachomatis* DNA after 1:100 dilution. One of the 3 male FCU which tested negative when diluted 1:100 was positive when tested after 1:10 dilution. There were insufficient samples of the other 3 FCU specimens (2 male FCU and 1 female FCU) which tested negative at 1:100 for retesting after a 1:10 dilution.

In our low-prevalence patient population, we found PCR inhibition rates of 7% for cervical swabs and 45% for urethral swabs. These rates of PCR inhibition strongly support the need to amplify and detect the IC DNA provided with the Roche PCR assay on all genital swab specimens. There may be regional differences in rates of PCR inhibition (1, 14, 16), and further studies with other patient populations in other geographic areas are needed. Few studies have investigated the inhibition in urethral swabs, and our rate of 45% is alarmingly high. The reason for the higher inhibition rate of urethral swabs compared to that of cervical swabs is unclear. Further studies with a larger number of urethral specimens are necessary to confirm these results.

Compared to swab specimens, PCR inhibition was detected in only 2.7% of female FCU and none of the male FCU. These results are consistent with other studies that have found a low rate of PCR inhibition in urine specimens (4, 6, 12). This is yet another reason why FCU are the preferred specimen type for PCR testing for diagnosis of chlamydial infection in asymptomatic men (15). Inhibition may be lower with FCU than that with genital swabs because of the dilution of inhibitory factors in a urine specimen or the fact that these specimens are stored at 4°C prior to processing.

Although heme may be inhibitory to PCR, we did not find that blood in specimens resulted in significantly higher PCR inhibition, in agreement with other studies (8, 16). In contrast, leaving the swab in STM results in a significantly higher PCR inhibition rate. It appears that prolonged exposure of the swab to STM results in PCR inhibition, although the exact mechanism by which this occurs has not been elucidated (1). The manufacturer recommends that swabs be removed and discarded after inoculation of the STM. Unfortunately, physicians may not always comply with this, since they are more accustomed to leave a swab in a transport tube than to discard it.

Phenol-chloroform extraction, storage at 4°C, and dilution of the processed specimen after storage were all effective in eliminating PCR inhibition from the majority of specimens. There was no significant difference between diluting the specimen 1:10 or 1:100 in eliminating inhibition. Verkooyen et al. reported that pretreatment at 4°C was ineffective in eliminating PCR inhibition (16). However, they pretreated the specimen at this temperature for only 10 min, in contrast to storing the specimen for ≥1 day at 4°C, as was done in our study. Storage of the specimen at −70°C was the least-effective method, especially for urethral specimens. Reports of positive specimens testing negative after freeze-thawing should also discourage use of this approach to eliminate PCR inhibition (16).

It has been observed in several studies that specimens that were initially falsely negative for *C. trachomatis* DNA became positive after storage and repeat testing (1, 2, 8, 9). Thus, substances that inhibit PCR may be temperature sensitive or become inactive over time, since storage of specimens at low temperatures or even heating to 95°C has allowed for resolution of inhibition (2, 8, 9, 10, 16).

The major potential disadvantage of using dilution to eliminate PCR inhibition is that this may result in missing a *C. trachomatis*-positive specimen containing low numbers of target DNA copies, especially with urine specimens. Diluting the specimen 1:10 is as effective in eliminating inhibition and is probably preferred to a 1:100 dilution. However, even at this dilution, a positive specimen may become negative, as has been previously reported (16).

Storage at 4°C and 1:10 dilution of the processed specimen are methods that can be easily incorporated into a clinical laboratory for the treatment of specimens that demonstrate PCR inhibition. Storage at 4°C may be preferred to dilution,

since this involves less manipulation of the specimen and would not reduce the sensitivity of the assay, especially with urine specimens. Perhaps incorporating one of these methods into the processing of all swab specimens routinely before initial testing will reduce the inhibition rate. Our preliminary studies with processing cervical swab specimens and storing them at 4°C overnight before testing on the next day have found a PCR inhibition rate of <3%. However, the optimal duration of storage of the processed specimen at 4°C prior to testing remains to be determined. Other alternatives that have been reported to yield a lower rate of PCR inhibition by the Roche assay include the use of 2-SP culture transport medium or the use of a dry swab specimen (7, 15, 16).

In summary, PCR inhibition is rare with FCU and infrequent with endocervical swabs but occurs frequently with urethral swabs. The routine detection of the IC DNA provided by the Roche assay is recommended, especially for genital swab specimens.

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