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Pooling of Clinical Specimens Prior to Testing for *Chlamydia trachomatis* by PCR Is Accurate and Cost Saving

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The accuracy and cost savings of pooling specimens prior to testing for *Chlamydia trachomatis* by PCR were evaluated with genital and urine specimens ($n = 2,600$). There was a 60% reduction in tests without significant loss of accuracy. The efficiency of pooling vaginal swabs is demonstrated for the first time.

Nucleic acid amplification tests, although sensitive and specific, are expensive. Acceptable accuracy and cost savings have been demonstrated by pooling urine and pooling endocervical specimens to detect *Chlamydia trachomatis* (1–9), but we are not aware of any studies testing pooled vaginal swabs. The aim of this study was to determine the accuracy and cost savings associated with pooling vaginal swabs as well as endocervical swabs and urine specimens for the detection of *C. trachomatis* by PCR.

All genital swabs and urine specimens sent to a large hospital-based laboratory over a 3-month period were tested individually and in pools of five. This pool size is consistent with other studies and was considered likely to maximize savings without significantly compromising sensitivity in our population. On receipt in the laboratory, individual specimens were processed and tested the following working day in accordance with the manufacturer's instructions (Roche Diagnostics Systems). Sixty percent of urine specimens and a small percentage of genital swabs were stored at −20°C for 1 to 4 weeks prior to pooling. Frozen specimens were thawed, pooled, and tested on the same day. One-hundred-microliter aliquots from each processed swab were combined to create pools of five, and 50 μl of this was amplified. One-hundred-microliter aliquots from each unprocessed urine specimen were combined in pools of five and processed, and 50 μl of the processed pool sample was amplified.

Specimens were considered positive if the absorbance at 660 nm was ≥0.8 and the absorbance for the internal control was ≥2. All pools and 50% of the individually tested specimens included internal controls. When a pool was positive, all individual samples were retested the next working day to identify the positive specimen(s). Specimens testing negative in the pool were deemed negative in the presence of a positive internal control. Specimens from pools presumed to be inhibited (i.e., a negative internal control) were retested individually. Inhibited individual specimens were diluted 1:10 and retested the next working day. All individual and pooled specimens were retested if the results were discrepant.

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We compared the accuracy of the PCR test with the pooled and individually tested specimens and calculated 95% confidence intervals (Stata Statistical Software, Release 7; Stat Corporation, College Station, Tex.). We compared inhibition rates for pooled specimens with present laboratory inhibition rates, as internal controls are now included in all tests.

Cost savings attributable to pooling were calculated by using normal laboratory procedures (individual testing plus reflex testing of positive, inhibited, and equivocal tests) as the baseline. Analyzed elements were the costs of reagents and other consumables plus the time taken by the technologist to perform all tests (pooled, individual, and reflex tests).

Seven-hundred fifteen vaginal swabs (143 pools), 885 endocervical swabs (173 pools), and 1,000 urine specimens (200 urine pools) were tested, of which 17, 34, and 63 specimens, respectively, were retested because they were positive, inhibited, or both. A total of 117 out of 2,600 (4.5%) specimens were positive for *C. trachomatis*.

There were no statistically significant differences in detection rates between specimens tested individually or in a pool (McNemar's test P value of 0.5 for vaginal and 0.6 for endocervical swabs; urine results were concordant). The pooling protocol correctly classified 17 of the 19 positive vaginal swabs detected by individual testing and all of those found to be negative (696). There were 43 positive endocervical swabs, 4 of which were discrepant. Of these, three were incorrectly classified as negative by the pooling protocol and one was identified in the pool testing but not the individual testing. There were no equivocal tests. Discrepant results were considered true-positive results following reflex testing. Differences in test accuracy are demonstrated in Table 1. There are no significant differences in inhibition rates between pooled and comparison specimens, although the difference between the pooled vaginal swabs and the comparison specimens (4.2% versus 10.4%) approaches statistical significance (Table 2).

Pooling the specimens resulted in a 60% reduction in the number of tests performed (1,114 versus 2,769 tests). A 39% reduction in total costs consisted of a 43% reduction in the quantity of reagents used, a 55% reduction in the costs of other consumables, and a 26% reduction in technologist's time.

Consistent with other studies, the pooling protocol was accurate and cost saving for urine specimens and endocervical

TABLE 1. Accuracy of PCR in detecting *C. trachomatis* in pooled compared to individual specimens

PCR criterion[a]	95% Confidence interval (range) for:		
	Vaginal swabs	Endocervical swabs	Urine specimens
Sensitivity	89.5 (81.2–93.9)	92.7 (86.2–96.8)	100 (95.6–100)
Specificity	100 (95.6–100)	99.9 (94.9–100)	100 (95.6–100)
PPV	100 (95.6–100)	97.4 (91.2–99.3)	100 (95.6–100)
NPV	99.7 (96.6–100)	99.6 (96.4–100)	100 (95.6–100)

[a] PPV, positive predictive value; NPV, negative predictive value.

swabs. Pooling vaginal swabs also resulted in cost savings without significant loss of accuracy.

Freeze-thawing did not appear to affect inhibition rates or to significantly reduce target DNA concentrations, as demonstrated by the complete concordance of positives detected in the both the individual and pooled urine specimens. Despite freeze-thawing and the dilutional effect of pooling, there were still enough inhibitors present in three of the urine pools to mask a positive result, highlighting the importance of incorporating an internal control.

A major limitation of our study is that an exact comparison of pooling and individual testing was not possible, because individually tested and pooled specimens were treated differently (freeze-thawing, use of internal controls). In addition, although vaginal swabs are increasingly used and are demonstrably more acceptable to patients (10), their use with the Roche PCR kit has not yet been approved by either the manufacturer, the U.S. Food and Drug Administration, or the Therapeutic Goods Administration in Australia.

A significant proportion of chlamydia tests are now combined with tests for gonorrhoeae, which has a much higher rate of false-positive results. More studies are needed to determine the cost savings, if any, in these combined tests.

Pooling could be introduced into the diagnostic setting to reduce costs as long as the prevalence of chlamydia or inhibitors of PCR were not too high. Kacena et al. modeled a 39% reduction in costs even with a prevalence of 8% (3). If pooling were introduced as standard practice, laboratories would need to meet the standards set down by national testing authorities, the technique would have to be acceptable to government funding agencies, and vaginal swabs would require approval for use with the manufacturer's PCR testing kit.

TABLE 2. Inhibition rates in pooled and comparison specimens[a]

Specimen type	Pooled test (%)	Comparison test (%)	Chi square test *P* value
Vaginal swabs	6/143 (4.2)	23/222 (10.4)	0.06
Endocervical swabs	10/173 (5.8)	12/160 (7.5)	0.8
Urine	21/200 (10.5)	26/423 (6.1)	0.8

[a] Present laboratory inhibition rates.

The future of chlamydia control depends on introducing widespread, targeted screening of at-risk populations. The savings associated with pooling may help to make this possible, even in resource-poor settings.

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