

## Pooling Cervical Swabs and Testing by Ligase Chain Reaction Are Accurate and Cost-Saving Strategies for Diagnosis of *Chlamydia trachomatis*

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**Specimen pooling to achieve efficiency when testing urine specimens for *Chlamydia trachomatis* nucleic acids has been suggested. We pooled endocervical swabs from 1,288 women and also tested individual swabs by ligase chain reaction (LCR). Out of 53 positive specimens, pools of 4 or 8 specimens missed two positives, providing 96.2% accuracy compared to individual test results. Dilution and positive-control spiking experiments showed that negative specimens with inhibitors of LCR in the pool reduced the signal. Conversely, two extra positives, detected only through pooling, were negative by individual testing but became positive after storage, suggesting that fresh positive specimens with labile inhibitors may be positive in a pool because of dilution of inhibitors. For this population of women with a 4% prevalence of *C. trachomatis* infection, substantial savings in cost of reagents (55 to 63%) and technologist time (50 to 63%) made pooling strategies a desirable alternative to individual testing.**

The impact of *Chlamydia trachomatis* infections on adult and infant populations has been well documented (1, 11). The demand for diagnostic tests for symptomatic patients and screening of asymptomatic populations is of great interest in laboratory medicine. This has resulted in the development and evaluation of many detection methodologies. Based on a number of studies comparing the sensitivities and specificities of culture, enzyme immunoassay, nucleic acid hybridization, and nucleic acid amplification (NAA) techniques, it is now generally accepted that NAA techniques are presently the most accurate assays for the detection of *C. trachomatis* (1, 3, 12, 13). Comparative studies have suggested that although first-void urine specimens or self-collected vaginal swabs may be ideal specimens for screening purposes, cervical swabs may still provide higher sensitivity rates (2). The higher cost of NAA technologies has limited their implementation for routine testing. Test costs may be decreased and accuracy may be maintained by pooling of urine specimens with retesting of individual samples from positive pools (5, 6, 9). However, there has been a concern about the role of amplification inhibitors in first-void urine (4, 7) and their role in the pooling of specimens. Few studies have been performed by pooling endocervical swabs.

We conducted this study to determine the accuracy and cost saving involved in pooling four or eight endocervical swabs collected for *C. trachomatis* testing by ligase chain reaction (LCR) technology.

### MATERIALS AND METHODS

A total of 1,288 endocervical samples collected from individuals attending a women's clinic were tested for *C. trachomatis* by use of the

Abbott LCx Probe System. All samples were collected using LCx swabs, received within 24 h of collection, held at 4°C, and tested within 48 h.

We created pools of four ( $n = 322$ ) and eight ( $n = 161$ ) cervical swabs. The swabs were sequentially numbered (i.e., 1 through 8). Aliquots of 100  $\mu$ l from each of the eight patient samples were transferred into each of three separate pooling tubes. This resulted in the formation of one pool of eight and two pools of four. The pools were vortexed to mix the contents. Each specimen and pool was then tested as described in the LCx package insert. Results were tabulated using the standard specimen-to-cutoff ratio (1.0) and with a specimen-to-cutoff ratio lowered by 0.2.

When the results achieved by individual testing did not agree with the pool results, tests were repeated on all of the specimens within the particular pool (reflex testing). For two pools that had low positive values and negative individual results, we performed PCR testing and direct fluorescent-antibody assay (DFA). Agreement of one of the two confirmatory tests with the LCx result was considered the correct result. A PathoDx *C. trachomatis* DFA kit (Diagnostic Products Corporation, Los Angeles, Calif.) result of one distinct elementary body (EB) was considered positive. We also used an in-house PCR with plasmid primers for confirmation (8). The numbers of true positives and negatives were set following these confirmatory tests.

A spiking experiment was performed to determine the effect of inhibitors of LCR in two negative pools of eight which contained positive individual specimens. Each individual original sample which went into these pools was spiked with a positive *C. trachomatis* L2 strain containing at least one infectious unit (4). Each undiluted and

TABLE 1. Accuracy of individual testing versus two pooling strategies for detecting *C. trachomatis* in endocervical swabs by LCR

Test strategy	% Positive (no./total) <sup>a</sup>	% Negative (no./total)
Individual	96.2 (51/53)	100 (1,235/1,235)
Pool of 4	96.2 (51/53)	100 (1,235/1,235)
Pool of 8	94.2 (50/53)	100 (1,235/1,235)

<sup>a</sup> The total number of positives was 53 as determined by confirmation of all specimens found positive by the three testing strategies.

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TABLE 2. Discrepant results obtained for cervical swabs tested individually and in pools of four and eight by LCx

Specimen no.	Test result (sample rate/cutoff rate) <sup>a</sup> for:		
	Individual test	Pool of 4	Pool of 8
BP-8765	POS (1,011.1/426.1)	POS (583.5/426.1)	NEG (252.7/426.1)
46-384519	NEG (184.4/577.1)	POS (895.1/577.1)	POS (893.1/577.1)
	POS (539.6/462.2) <sup>b</sup>		
BP-8904	NEG (463.9/577.1)	POS (895.1/577.1)	POS (893.7/577.1)
	POS (672.7/462.2) <sup>b</sup>		
BP-9082	POS (660.9/448.3)	NEG (11.1/448.3)	NEG (160.7/448.3)
BP-16300	POS (2,043.0/654.6)	NEG (13.7/654.6)	NEG (14.0/654.6)

<sup>a</sup> The rate was calculated as described in the manufacturer's package insert (as counts per second per second) and printed from the LCx analyzer. POS, positive; NEG, negative.

<sup>b</sup> The sample was retested after 24 h at 4°C.

1:4-diluted sample that was spiked and a 1:4-diluted sample that was not spiked were tested in the LCx.

Cost savings were calculated for the following three scenarios: (i) individual testing, (ii) pooling of four or eight specimens followed by individual testing, and (iii) pooling of eight specimens followed by testing two pools of four before individual testing. Since costs of reagents and technologist time vary from laboratory to laboratory or in different countries, we calculated percent savings in these two categories at our rate of prevalence.

## RESULTS

The prevalence of *C. trachomatis* in the population tested in this study was 4.1% (53 of 1,288). Individual testing identified 51 (96.2%) of the 53 positives and 100% of the negatives (Table 1). The pools of four, using the manufacturer's cutoff value, detected 51 (96.2%) of 53 positives, and the pools of eight found 50 (94.2%) of 53 positives. Reduction of the cutoff by 0.2 of the value did not increase the number of positive pools.

There were five discrepant results which were reexamined (Table 2). One specimen (BP-8765) was positive when tested

individually. The pool of four containing BP-8765 was positive, but the pool of eight was not. The pools of four and eight which contained specimens 46-384519 and BP-8904 were positive, but the specimens were negative in the original individual tests. Both samples contained EBs in very low numbers (one and two, respectively) as determined by DFA staining and were scored as low positives (scores of 539.6 and 672.7 when the cutoff value was 462.2) on repeat testing after overnight storage at 4°C. Two other samples (BP-9082 and BP-16300) were positive on individual testing but negative in their pools of four and eight.

To determine whether the two individual positives (BP-9082 and BP-16300 in Table 2) were negative in the pools of four or eight because of the presence of inhibitory specimens in the pool, we performed dilution and spiking experiments, which are summarized in Table 3. Each pooled specimen was tested individually, undiluted and at a 1:4 dilution with the addition of a *C. trachomatis* spike. A third tube without the spike at a dilution of 1:4 was also tested. The data show that specimen BP-16303 contained inhibitors which presumably masked the positive specimen BP-16300 in the pool, and the inhibitory specimen still had activity at a dilution of 1:4. Similarly, the pool containing specimen BP-9082 contained an inhibitory specimen (BP-9086).

We calculated savings of reagents and technologists' time for three pooling strategies, which were (i) pools of four with the positive pools reflexed into individual testing of the four specimens, (ii) positive pools of eight reflexed into eight individual tests, and (iii) positive pools of eight reflexed to testing two pools of four, followed by individual testing of the positive pool of four.

Table 4 shows the cost savings created by a reduction in the number of tests performed in each pooling strategy versus individually testing all 1,288 swabs. By pooling samples in fours, a decrease in the number of tests created a saving of 60% on materials when the number reached 80 tests. Comparable saving on the eight-pool model was 55%. Reagent consumption in the third strategy decreased when a positive pool

TABLE 3. Detection of inhibitory specimens within pools of cervical swabs by individual dilution and spiking experiments for LCx Chlamydia

Specimen no.	Original LCx rate <sup>a</sup> for:				LCx rates after individual spiking with:			Inhibition
	Individual test	Pool of 4	Pool of 8	Run cutoff	<i>C. trachomatis</i> neat	<i>C. trachomatis</i> 1:4 dilution	No. <i>C. trachomatis</i> 1:4 dilution	
BP-16300	2,043.0	13.7	14.0	654.6	2,177.3	2,140.8	2,282.4	No
BP-16301	15.1				ND <sup>b</sup>	1,518.6	21.9	No
BP-16302	14.2				1,586.4	1,792.8	20.9	No
BP-16303	14.0				18.1	16.7	17.2	Yes
BP-16304	14.6	14.6			1,628.2	1,573	20.8	No
50-31229	14.0				1,685.5	1,560	18.5	No
50-31230	14.1				805.6	1,727.3	20.2	No
50-31231	16.5				22.5	1,357.9	19.5	Yes
BP-9082	660.9	11.1	160.7	448.3	ND	1,557.6	736.2	No
BP-9080	11.7				ND	1,634.7	22.2	No
BP-9081	10.3				ND	1,547.2	17.8	No
BP-9083	11.5				ND	1,647.2	291.8	No
BP-9084	10.8	11.2			ND	1,572	18.2	No
BP-9085	11.1				ND	1,621.2	19.4	No
BP-9086	11.2				18.1	1,096.8	18.8	Yes
BP-9088	11.2				1,895.7	1,499	18.1	No

<sup>a</sup> The rate was calculated as described in the manufacturer's package insert (as counts per second per second) and printed from the LCx analyzer.

<sup>b</sup> ND, not determined due to insufficient specimen for testing.

TABLE 4. Requirements for test reagents and technologist time for three LCx Chlamydia pooling strategies at a 4% prevalence rate

Individual testing		Pool of 4		Pool of 8		Pool of 8 to pools of 4	
No. of tests	Staff <sup>a</sup>	No. of tests (%) <sup>b</sup>	Staff (%) <sup>c</sup>	No. of tests (%)	Staff (%)	No. of tests (%)	Staff (%)
20	0.5	11 (55)					
80	1	32 (60)	0.5 (50)	36 (55)	0.5 (50)	30 (63)	0.5 (50)
160	2	64 (60)	1 (50)	72 (55)	1 (50)	60 (63)	1 (50)
320	4	128 (60)	2 (50)	144 (55)	2 (50)	119 (63)	2 (50)
640	8	256 (60)	4 (50)	288 (55)	4 (50)	237 (63)	3 (63)

<sup>a</sup> Staffing requirements in full-time equivalents (1 full-time equivalent = 7.5 h) from start to finish; the actual hands-on time equals 2 h. A second thermal cycler is required at a level of 80 tests and a second LCx analyzer is required at 160 tests.

<sup>b</sup> Savings as a percentage of individual testing, which is calculated by subtracting reagent consumption in the pooling strategy (*P*) from consumption in individual testing (*I*) and then dividing by *I*, i.e.,  $(I-P/I \times 100)$ .

<sup>c</sup> Savings of technologist time as a percentage of time required for individual testing.

of eight was tested as two pools of four, with individual testing restricted only to the positive pool. This strategy resulted in a reagent and staff cost reduction of 63% when the number of tests reached 640. There was a saving in technologist time of 50% with any of the pooling systems when 81 or more specimens were processed.

## DISCUSSION

To our knowledge, this is the first published report on the pooling of cervical swabs for the diagnosis of *C. trachomatis* infections using LCR. Krepel et al. (6) found in testing 1,220 urine specimens that individual testing missed two positives, whereas pooling in groups of four missed four positives; extra positives were found when the cutoff was lowered. Kacena et al. (5) reported a similar study comparing testing of individual urine specimens to testing pools of 4 or 10 urine specimens. Using a similar lowered signal-to-cutoff ratio in the LCx test, they found 100% accuracy with pools of 4 and 98.4% accuracy with pools of 10. Our cervical swab study showed diagnostic accuracy of 96.2% (51 of 53) by individual testing and testing in pools of four and 94.2% accuracy (50 of 53) by testing in pools of eight. Adjusting the LCx test cutoff in our study did not identify more positives. Peeling et al. (9) performed a urine pooling study using the Amplicor PCR test and showed that by pooling 370 archived urine specimens from asymptomatic men into groups of five, the strategy missed 1 of 19 positives. In our cervical swab pooling study, two specimens positive by individual testing were masked in their pools and two other specimens initially negative by individual testing were positive in their pools. A fifth specimen was positive individually and in its pool of four but was negative in its pool of eight.

Specimen pooling has been a successful strategy for testing sera for human immunodeficiency virus (10) and may be extended to other high-volume testing. Our study and those already published showed good ability of amplification assays to identify pools containing positive specimens. Our cervical swab study showed that two positive patients would have been missed by individual testing if pooling was not conducted. This may be due to inhibitors of amplification in the fresh individual specimens, which were presumably diluted as a result of pooling, enabling the pool to be positive. When the individual urine specimens from these pools were retested on the following day, they were repeatedly positive and contained EBs, suggesting that the inhibition was labile.

Pooling may place a positive specimen together with specimens which contain inhibitors. In our study this happened with at least two specimens that were positive on individual testing but were negative in a pool. By performing dilution and spiking experiments we showed that this was the case in pools of four

and eight. Thus, there appears to be a slight disadvantage to pooling due to inhibitors in a pool, which is offset by the emergence of positives in a pool because of the dilution of inhibitors in an individual specimen due to pooling. Mahony and coworkers (7) have published inhibitor rates for LCx and other amplification tests. This issue deserves more well-designed studies to enable appropriate recommendations on the need for internal controls. Alternative strategies involving dilution or delay of testing of the specimens may remove inhibitors. Additionally, two pools of eight had values slightly above the cutoff, yielding presumptive false-positive results. On reflex testing and performance of DFA and PCR, all individual swabs were negative, showing that pooling followed by individual testing would eliminate presumptive false positives.

Our study, on a population with a *C. trachomatis* prevalence of 4%, has shown that creating pools of four cervical swabs identified 96.2% of the positives and allowed a cost saving of 60% of reagent costs and 50% of technologist salaries. This cost saving is higher than the 44.5% saving shown by Krepel et al. (6) but is closer to the 57% shown in a PCR study on archived specimens (9). Kacena and coworkers (5) calculated a reduction in costs of 39% by pooling in fours in a population with 8% prevalence. Our reagent cost savings of 55% (going from pools of eight to individual tests), 60% (going from pools of four to individual tests), and up to 63% (going from pools of eight to pools of four before going to individual testing) are all calculated for a 4% prevalence of infection, and these could change as prevalence changes. The savings were realized as 80 or more tests were performed. Our calculations also showed 50 to 63% savings in technologist time due to the various pooling strategies, even with the extra handling time required to create pools. Pools of eight did not decrease technologist time significantly compared to pools of four; reflex testing of positive pools required more individual tests to be performed.

Reflex testing does create delays to the final report of a positive or negative result. Pooling may not allow optimal delivery of timely reports in certain settings, but generally, pooling cervical swab samples for detection of *C. trachomatis* by LCR can be accurate and provide substantial cost savings.

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