

Pooling of Urine Specimens for Detection of Asymptomatic *Chlamydia trachomatis* Infections by PCR in a Low-Prevalence Population: Cost-Saving Strategy for Epidemiological Studies and Screening Programs

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Pooling, in groups of five, of urine specimens from asymptotically infected men in a population with 4% prevalence, as determined by case finding, is 100% sensitive and specific and results in a 60.5% reduction in the number of tests needed. Pooling of urine specimens in groups of 10 for the estimation of population-based prevalence is 96.1% sensitive and 100% specific and saves 90% of the test costs.

It has been suggested that screening programs for the detection of asymptomatic *Chlamydia trachomatis* infections should be developed to reduce the burden of disease caused by *C. trachomatis* (3, 6, 8, 9). Two studies have been published concerning the pooling of urine specimens to reduce the costs associated with handling large amounts of samples for the detection of *C. trachomatis* (1, 7). The first study (7) used PCR on archived urine specimens from males, and the second study used ligase chain reaction (LCR) on fresh urine specimens from women (1); both showed high test sensitivity and cost-saving aspects. However, while lowering the cutoff value in the LCR was necessary to identify all positive pools (1), the first study acknowledged the need to validate the pooling strategy using fresh urine specimens (7). Members of our group recently showed that PCR performs better than LCR for the detection of asymptomatic *C. trachomatis* infections in urine specimens from both males and females (5).

Therefore, this study evaluated different characteristics of urine specimen pooling for the detection of *C. trachomatis* by PCR using fresh urine specimens from asymptotically infected males in a low-prevalence population. Two evaluations were performed, as follows: (i) estimation of the population-based *C. trachomatis* prevalence without testing *C. trachomatis*-positive pools and (ii) identification of all individual *C. trachomatis*-positive cases based on *C. trachomatis*-positive pools.

First-voided urine specimens ($n = 650$) were obtained from asymptomatic (defined as having no clinical symptoms or not having contacted a physician for possible urogenital complaints) Danish male military recruits and sent by air mail to the laboratory within 3 days (samples were at room temperature during transport) (4). Urine specimens were tested for *C. trachomatis* by PCR (Amplicor; Roche Diagnostic Systems, Basel, Switzerland). Specimens were tested individually, pooled in groups of five into 130 pools, and pooled in groups of 10 into 65 pools. Each sample received a laboratory access-

ion number, and consecutively numbered samples formed each pool.

Specimen preparation and *C. trachomatis* testing were performed according to the instructions of the manufacturer and as described previously (5). For pooled urine specimens the same procedure for *C. trachomatis* detection was used except that, for pools of five urine specimens, instead of 0.5 ml of urine, five 100- μ l samples of urine, well mixed, were used (0.5 ml). For pools of 10 urine specimens, 10 50- μ l samples were used. For both individual and pooled sample testing, the visibility of the urine pellet after centrifugation was monitored. Cost analysis was based on the number of tests saved by comparing individual sample testing and pooling of urine specimens by 5 or by 10, including retesting of the individual urine samples of the *C. trachomatis*-positive pools. The estimation of the population-based *C. trachomatis* prevalence (maximum likely prevalence [p]) was performed as described previously by Kline et al. (2).

Estimation of the population-based prevalence. Of the 650 first-voided urine specimens, 26 (4%) were positive for *C. trachomatis* by PCR, and results were confirmed by retesting. Assessment of the *C. trachomatis* prevalence in a population is a prerequisite for cost-benefit analysis to determine if screening in a certain population should be initiated. When urine specimens were pooled by 5 and by 10, 25 out of 130 pools and 23 out of 65 pools were *C. trachomatis* positive, respectively. In both pooling strategies, two pools (containing four different *C. trachomatis*-positive samples) were grey zone (between 0.2 and 0.8) and by retesting were *C. trachomatis* positive and grey zone, respectively. Both were considered *C. trachomatis* positive as defined by the manufacturer's instructions. The population-based *C. trachomatis* prevalence could be estimated without testing all *C. trachomatis*-positive pools for the individual *C. trachomatis*-positive samples. As shown in Table 1, p was 4.2% (95% confidence interval [CI], 2.54 to 5.82) when urine specimens were pooled by 5 and 4.3% (95% CI, 2.51 to 6.03) when urine specimens were pooled by 10. The *C. trachomatis* prevalence as determined by individual testing was 4.0%, which is within the 95% CIs of the estimates of *C. trachomatis* prevalence. Pooling of urine specimens by 5 or 10 reduced test costs by 80% (132 versus 650) or 90% (67 versus 650), respec-

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TABLE 1. Data for urine specimens collected and pooled for the detection of *C. trachomatis* in an asymptotically infected male population

Characteristic	Data for specimens that were:		
	Tested Individually	Pooled	
		By 5	By 10
No. of urine specimens	650	650	650
No. of samples/pool		5	10
No. of pools		130	65
No. of <i>C. trachomatis</i> -positive pools		25	23
No. of <i>C. trachomatis</i> -positive samples	26	26	25
Sensitivity (%)		100	96.1
Specificity (%)		100	100
Prevalence (%)	4.0	4.2 ^a	4.3 ^a
95% CI		2.5–5.8	2.5–6.0
Cost savings ^b for:			
Population-based prevalence determination (%)		80	90
Individual case finding (%)		60.5	54

^a Estimated by *p* (1).

^b Cost savings estimates are based on the reduced number of tests.

tively. These data indicate that pooling by 10, although resulting in a sensitivity of slightly less than 100%, could be used to determine the *C. trachomatis* prevalence in large populations in a strategy that is quick and cost saving compared to individual sample testing.

Case finding based on *C. trachomatis*-positive pools. When urine specimens were pooled by 5, individual testing showed that 24 pools contained one *C. trachomatis*-positive case each and 1 pool contained two *C. trachomatis*-positive cases, adding up to the identification of all 26 *C. trachomatis*-positive males. This makes the pooling of urine specimens by 5 100% sensitive and 100% specific (Table 1). When urine specimens were pooled by 10, individual testing showed that 22 pools contained one *C. trachomatis*-positive case each and 1 pool contained three *C. trachomatis*-positive cases, adding up to the identification of 25 of the 26 *C. trachomatis*-positive males. This makes the pooling of urine specimens by 10 96.1% sensitive and 100% specific (Table 1). Peeling et al. (7) showed that pooling by five of archival male urine specimens was 94.4% sensitive as determined by PCR. Kacena et al. (1) showed that pooling urine specimens from females by 4 was 100% sensitive, while pooling by 10 was 98.4% sensitive as determined by LCx.

By pooling 5 or 10 urine specimens, 60.5% (257 versus 650) and 54% (297 versus 650) of the test costs were saved, respectively. Peeling et al. (7) showed that five-specimen pooling resulted in a cost savings of 57%. Kacena et al. (1) included test price (LCx), technician time, laboratory consumables, and the *C. trachomatis* prevalence (4%) in the cost analysis and showed that pooling specimens in groups of 5 would result in a 49% cost savings. When we included these additional variables in our setting, the total cost savings of a five-specimen pooling strategy was 61.9% compared to individual testing. These data show that case finding by pooling five urine specimens in this population with a *C. trachomatis* prevalence of 4% is a sensitive and cost-saving approach.

Besides the cost-saving aspects, two additional favorable effects of pooling urine specimens were observed. Of the urine specimens tested individually, 12.5% (81 out of 650) had an

invisible pellet after centrifugation of a 0.5-ml urine specimen plus 0.5 ml of wash buffer. This made correct removal of the supernatant difficult. Furthermore, three urine samples showed inhibition, as determined by the inability to amplify the internal control included in the Amplicor assay. This corresponds to an inhibition rate of 0.5%. In contrast, all pooled urine specimens, whether pooled by 5 or by 10, had a visible pellet after centrifugation ($P < 0.0001$, versus individual testing), which made the removal of the supernatant easier. Furthermore, no inhibition was observed in the pooled urine specimens. This decrease of inhibition was probably due to dilution of the inhibitory factors. However, when urine specimens were tested individually, pooled by 5, and pooled by 10, the number of grey-zone values for the *C. trachomatis*-positive pools increased from 0% (0 of 650) to 1.5% (2 of 130) and 3% (2 of 65), respectively. However, it was not necessary to change the sample cutoff value for the pooled specimens, a change that Kacena et al. (1) found to be necessary for the LCR, and five-specimen pooling was still 100% sensitive.

Two possible disadvantages should be acknowledged. Although urine specimens stay stable up to 1 week at room temperature (4), special laboratory logistics are needed to test all individual samples in the *C. trachomatis*-positive pools within 1 week. Alternatively, urine specimens could be stored at 4°C. Secondly, the pooling strategy might encounter the difficulty of being "off label" in some areas. Most likely, governmental screening programs will be able to use the pooling strategy, but obtaining approval for specific diagnostic screening programs could be difficult. In conclusion, this study showed that the implementation of the pooling strategy for urine specimens for the detection of *C. trachomatis* could have a major impact on the feasibility of screening programs.

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