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Pooling Cervical Swabs for Detection of *Chlamydia trachomatis* by PCR: Sensitivity, Dilution, Inhibition, and Cost-Saving Aspects

Only two studies have investigated the pooling approach for cervical scrapes. In a small study, Lisby et al. (3) showed that pooling in groups of 5 was 100% sensitive and reduced costs. Recently, Kapala et al. (2) showed in a large study using a ligase chain reaction (LCR) that the pooling strategy is a desirable alternative to individual testing. However, both studies demonstrated the need for additional studies on dilution, inhibition, and internal controls. A pooling approach for a large research study was investigated, with special attention to inhibition and dilution. Beta-globin PCR was used to monitor inhibition and the presence of human cells followed by an in-house *Chlamydia trachomatis* PCR which is more sensitive (4) than PCR (Roche) and LCR (Abbott) and allows for much higher through-put for considerably less money.

Initially, 500 randomly selected cervical scrapes were used to optimize the pooling strategy. Cervical specimens were pooled in groups of 5 by mixing 50 μl of each well-vortexed cervical sample in one tube. For all 500 individual samples and all 100 pools of 5, *C. trachomatis* and beta-globin PCRs were performed using 20, 10, or 5 μl as input in the PCR. PCRs and enzyme immunoassays were performed as described previously (1, 5). The results are shown in Table 1. Reducing the input in the PCR from 20 or 10 μl to 5 μl resulted in a large reduction of inhibition, as shown by beta-globin PCRs, especially in the pooled samples. Two additional *C. trachomatis*-positive pools, compared to individual testing, were found (10 and 5 μl of input). DNA isolation (High Pure PCR template preparation kit; Roche Diagnostics Corporation) from the beta-globin PCR- and *C. trachomatis* PCR-negative individual samples identified the two missed samples.

Second, the optimized pooling strategy was evaluated for 750 new cervical scrapes. Results are shown in Table 1. Pooling identified two samples in addition to those identified by individual testing. In addition, one sample was positive only in the individual testing but could not be confirmed and most likely is a sample with a very low titer. All pooled samples (*C. trachomatis* positive, $n = 38$) were further diluted to 10-, 50-, and 100-fold in a background of *C. trachomatis* PCR-negative and beta-globin PCR-positive cervical scrapes. All *C. trachomatis*-positive samples were detected when diluted 10-fold, 95% (36 of 38) were detected when diluted 50-fold, and 74% (28 of 38) were detected when diluted 100-fold. The high sensitivity even after further dilution is most likely due to the high number of *C. trachomatis* particles (1,000 to 10,000) per inclusion per human cell in combination with a high-copy target for the PCR (10 plasmid copies per particle), resulting in a sensitive PCR and pooling approach. Considering the discrepant sample as a true positive sample, individual testing was 95% sensitive (42 out of 44) and pooling was 98% sensitive (43 out of 44). The cost-saving aspect of pooling was considerable: in comparison to the testing of individual samples by *C. trachomatis* and beta-globin PCR, resulting in a total of 1,500 tests (Table 1, using 5 μl of input [2 × 750]), by the pooling strategy, 150 pools of 5 can be tested for *C. trachomatis* and the *C. trachomatis*-positive pools ($n = 38$) can be further tested using the individual samples ($n = 190$ [38 × 5]), reducing the number of tests by 77%.

TABLE 1. Optimization of pooling in groups of 5 for *C. trachomatis* PCR using cervical scrapes

Input (μl)	No. of PCR-positive samples by:							
	Chlamydia PCR				Beta-globin PCR			
	Individual samples ($n = 500$)	Samples pooled in groups of 5 ($n = 100$)	Individual samples ($n = 750$)	Samples pooled in groups of 5 ($n = 150$)	Individual samples ($n = 500$)	Samples pooled in groups of 5 ($n = 100$)	Individual samples ($n = 750$)	Samples pooled in groups of 5 ($n = 150$)
20	18	12[a]			413	88		
10	20	22[a,b]		464	100	100		
5	22	24[a,b]	42[c]	43[c,d]	490	100	ND[e]	150

[a] One pool contained two individual *C. trachomatis* samples.
[b] The two additional *C. trachomatis*-positive samples found by pooling showed inhibition for PCR in individual testing. DNA isolation from the individual samples confirmed *C. trachomatis* positivity.
[c] Two additional *C. trachomatis*-positive samples found by pooling showed inhibition for PCR in individual testing. DNA isolation from the individual samples confirmed *C. trachomatis* positivity. One sample was only found by individual testing, and DNA isolation of the pool did not result in *C. trachomatis* positivity in the PCR.
[d] Five pools contained two individual *C. trachomatis* samples.
[e] ND, not done.

In conclusion, our results and Kapala's results are separate but complementary, demonstrating that the pooling strategy is highly sensitive and cost-saving, with good dilution and inhibition characteristics, and is a strategy which should be implemented in screening settings. These results should be an incentive for pharmaceutical companies to evaluate this strategy, which could also potentially be used in diagnostic settings.

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