Pooling urine samples for PCR screening of *C trachomatis* urogenital infection in women

EDITOR,—Selective or universal screening for *Chlamydia trachomatis* infections has been sugested by the World Health Organization as a primary prevention strategy.[1]

The improved sensitivity of the nucleic acid amplification assays for the detection of *C trachomatis* allows the use of urine samples, suitable for screening programmes. However, these commercial assays are expensive, which make them disadvantageous for this purpose.

Therefore, some authors have recently evaluated the accuracy and cost saving of different urine pooling strategies using polymerase chain reaction (PCR) and ligase chain reaction (LCR) tests for the screening for genital *C trachomatis* infections, reporting very encouraging results.[2–5] As the pooling strategies need individual retesting of each component of a positive pool, in order to identify the positive samples the cost saving inherent to these strategies are prevalence and pool size dependent. For this reason, pooling may be particularly suitable when applied to low prevalence populations. On the other hand, a high number of urine samples per pool may yield a decreased sensitivity because of the dilution effect associated with pooling. Peeling *et al* and Kacena *et al* have put forward a mathematical formula to estimate the number of pools that are likely to be positive given a selected pool size and population disease prevalence.[2 3] Thus, it is possible to estimate the reduction on the number of tests required for a pooling strategy compared with individual testing.

The objective of this study was to evaluate a pooling urine samples strategy for screening urogenital chlamydial infection by PCR testing.

In all, 330 processed first catch urine samples (FCU) from women attending general practice clinics in Lisbon (from August 1999 to February 2000) were pooled by five into 66 pools. Pools and individual specimens were simultaneously tested using the Amplicor PCR test, according to the manufacturer's

Table 1 Distribution of positive samples

	"+" Pools (12)	*Equivocal pools (4)**	*"−" Pools (50)*
"+" Samples (17)	13	4	0

*Confirmed as positive pools.

instructions. Equivocal results analysis (>0.2 OD, <0.8 OD) was resolved by reprocessing original samples and by retesting both pooled and individual specimens by Amplicor PCR assay.

The results are summarised in table 1. The calculated prevalence was 5.2% (17/329). The dilution effect associated with the pooling strategy did not have any effect on either the sensitivity or specificity of the Amplicor PCR test (both 100%) and also solved the problem of PCR inhibitory substances in urine specimens (0% compared with 3.6% of individual testing). One FCU specimen was repeatedly inhibited and was excluded.

The choice for a 5× size pool model was based on the highest potential cost saving for the estimated prevalence of the studied population, according to Peeling *et al* and Kacena *et al*.[2 3] According to the number of tests required using pooling and individual testing (166 and 346, respectively) the cost saving was 52% compared with the 56% obtained using the mathematical formula. The main reason for this minor difference is that the formula does not take into account the inhibited and equivocal results requiring further sample testing.

Despite the low number of studies concerning urine pooling strategies, the results obtained so far suggest that pooling FCU samples can be useful for epidemiological studies and for screening programmes.

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J P GOMES
M A FERREIRA
Bacteriology Department, National Institute of Health, Lisbon, Portugal

A BRITO DE SÁ
Institute of Preventive Medicine, Lisbon Medical School, Lisbon, Portugal

M A CATRY
Bacteriology Department, National Institute of Health, Lisbon, Portugal

Correspondence to: João Paulo Gomes, Centro de Bacteriologia, Instituto Nacional de Saúde, Av Padre Cruz, 1649–016, Lisboa, Portugal

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