

## Pooled specimens for *Chlamydia trachomatis*: new approach to increase yield and cost efficiency

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**SUMMARY** Pooled specimens from the urethra and cervix accounted for 97% of 101 positive chlamydial isolations in 332 women, and this yield compared favourably with the individual yield from either the urethra (77%) or the cervix (88%). Pooling specimens caused no apparent increase in toxicity to the cell culture system. These results indicate the advantages, in terms of higher yield and no higher cost, of combining the urethral and cervical specimens in one container.

### Introduction

Despite refinements in laboratory procedures for the isolation of *Chlamydia trachomatis*, the collection of specimens remains an important factor influencing ultimate yields. The intracellular location of the organism means that a good harvest of epithelial cells is necessary to ensure acceptable results.<sup>1</sup>

Workers have claimed substantial increases in the numbers of diagnoses of female genital tract infections after multiple site testing.<sup>2</sup> This approach, however, increases the cost of an investigation whose availability is already severely restricted by cost (because the cell culture methods used are labour intensive). It is therefore surprising that studies of the potential advantages of processing pooled specimens from different sites have not been published.

In theory, a pooled specimen from the urethra and cervix might be expected to yield as much information as two separate specimens but at half the cost. The increased number of infectious particles in the pooled specimen might even result in an increased overall yield. Alternatively, enhanced toxicity to the cell culture system might make this method counter-productive. We therefore designed this study to explore these possibilities.

### Patients, materials, and methods

The study was performed on new and returning women

patients attending the sexually transmitted disease (STD) clinic at the General Hospital, Birmingham, from May 1985 to April 1986. The following women were excluded from the study: those who had taken antibiotics in the previous four weeks, were prepubertal, postmenopausal, or pregnant, or who had undergone hysterectomy. Informed consent was obtained from all the patients included in the study, and they were examined by one author only (ARGM). The samples were taken in the sequence shown in table I.

TABLE I Sequence of samples taken from and tests performed for each patient

1	Urethra	- Gram film and culture for <i>Neisseria gonorrhoeae</i> - Culture for <i>Chlamydia trachomatis</i> (individual specimen or pooled with specimen from cervix*)
2	Cervix	- Exfoliative cytology - Gram film and culture for <i>N gonorrhoeae</i>
3	Vagina	- Gram film - Wet film and culture for <i>Trichomonas vaginalis</i> - Amine and pH tests
4	Cervix	- Culture for <i>C trachomatis</i> (second individual specimen or pooled with specimen from urethra*)
5	Rectum	- Culture for <i>N gonorrhoeae</i>
6	Pharynx	- Culture for <i>N gonorrhoeae</i> (if fellatio practised)
7	Urine	- Urine analysis (N-Multistix)
8	Blood	- Serological tests for syphilis
9	Ulcers	- Culture for herpes simplex virus - Dark ground microscopy

\* Randomisation code decided order in which urethral and cervical specimens were first tested individually or together.

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**SPECIMENS FOR *C TRACHOMATIS***

Each patient was assigned a randomisation code that was used to decide which swab was to be placed first in the individual or pooled specimen container (fig 1). The urethral meatus was first wiped with a cotton wool ball soaked in saline. A sterile cotton tipped wooden swab moistened in the transport medium was then inserted into the urethra, rotated gently against the mucosa, and placed in the transport medium. The cervix was wiped with a dry cotton wool ball. A sterile cotton tipped wooden swab was then inserted into the endocervical canal, rotated against its wall a few times, and placed in the appropriate container.

**Storage and transport of specimens**

All chlamydial swabs were broken off into glass containers with 2 ml of the transport medium and stored at 4°C for less than 24 hours before inoculation. The transport medium was Eagle's minimum essential medium with Earle's salts, vitamins, non-essential amino acids, and 10% fetal calf serum.

**Isolation method**

After the transport medium was agitated on a vortex mixer, 1 ml was inoculated on to a monolayer of McCoy cells pretreated with 5-iodo-2'-deoxyuridine and grown on the flat base of a 16 x 100 mm glass tube. The monolayer tubes were centrifuged at 2000 RCF (relative centrifugal force) for one hour and incubated at 35°C for 48 hours. The presence or absence of chlamydial inclusions was shown by a fluorescence technique developed in this laboratory.

After the tissue monolayer had been washed carefully in phosphate buffered saline (PBS), the cells were scraped into the PBS residue and into 3 mm wells on a poly-tetra-fluo-ethylene (PTFE) multislide, allowed to air dry, and fixed in cold acetone. A volume of 4 µl of monoclonal antisera (Boots Celltech) was pipetted into each well. The slides were incubated for 30 minutes at 37°C, washed in PBS for five minutes, and

mounted in glycerol saline. We subcultured any tissue cultures that were unsuitable for microscopy because of a toxic effect or other factors.

**CULTURE FOR *NEISSERIA GONORRHOEAE***

Urethral, cervical, vaginal, and rectal swabs were inoculated on to Birmingham General Hospital vancomycin, colistin, and trimethoprim (VCT) medium and cultured as described previously.<sup>3</sup>

McNemar's test was used to compare the yields from individual and pooled specimens.<sup>4</sup>

**Results**

Of the 345 women examined, 13 were excluded from the study because of alkaline changes in the transport medium, disruption of the cell culture with *Trichomonas vaginalis* or herpes simplex virus, or labelling errors. *C trachomatis* was isolated from 101 (30%) of the remaining 332 women.

Figure 2 shows the yield from the individual and pooled specimens. *C trachomatis* was isolated from 98 (97%) pooled specimens compared with 89 (88%) single specimens from the cervix and 78 (77%) single specimens from the urethra. There were significant differences between the isolation rates from the pooled specimens compared with rates from single specimens from the cervix ( $p < 0.05$ ) and the urethra ( $p < 0.001$ ). Cultures were positive in 57 (34%) cases when pooled specimens were taken first and in 44 (27%) when single specimens were taken first. The difference was not significant.

*T vaginalis* was isolated from the chlamydial culture specimens from 4% (12/336) of women. By seeding the chlamydial specimens on to human embryo lung cells in separate tubes, herpes simplex virus was isolated from 3% (10/336) and cytomegalovirus from 2% (6/336) of women.

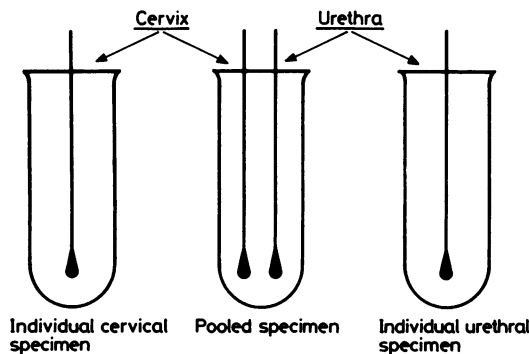


FIG 1 Definition of specimens taken from urethra and cervix

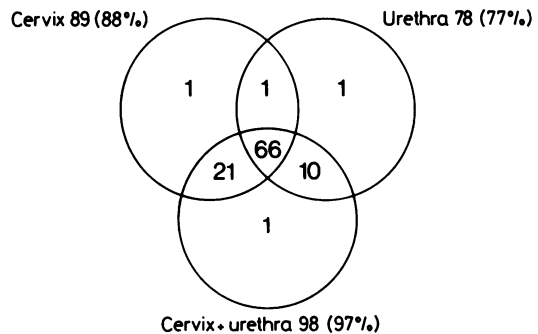


FIG 2 Isolation of *Chlamydia trachomatis* from individual cervical and urethral specimens and pooled specimens from 101 women

*C trachomatis* was isolated from 30/48 (63%) of women who were consorts of men with gonorrhoea and 28/58 (48%) of those whose sexual partners had non-gonococcal urethritis. Of the remaining 226 women, 43 (19%) gave positive results. *C trachomatis* was also isolated from 34 of the 59 (58%) women with coexistent culture proved gonorrhoea.

### Discussion

A significantly higher yield was obtained from the pooled specimens (97%) than from specimens from the cervix only (88%) ( $p < 0.05$ ) at no extra cost. Chlamydial isolation from over 30% of women attending STD clinics has been reported by only a few workers.<sup>5-7</sup> Our yield of 30.4% thus compares favourably with their studies and indicates the high sensitivity of the isolation method used in our study.

Dunlop *et al* isolated *C trachomatis* from 73% of women with a first swab, an additional 17% with a second swab, and 10% with a third.<sup>8</sup> They therefore suggested that two consecutive cervical swabs were preferable to a swab each from urethra and cervix, if facilities were available to take two chlamydial cultures per patients. Despite this, Munday *et al* suggested that the lower sensitivity of the isolation method used by Dunlop *et al* might have been responsible for the differences in the yield from consecutive cervical swabs.<sup>6</sup> In our study we took two consecutive specimens from the urethra and cervix of each woman and did not find any significant differences in yield, whatever the order of taking the swabs. Furthermore, Dunlop *et al* attributed the increased efficiency of the last two swabs to the cleaning effect of the first swab, which removed mucus from the cervix.<sup>8</sup> We performed cervical cytology before taking chlamydial swabs, which according to Mårdh *et al* might have cleaned the cervix.<sup>9</sup> Mucus was therefore unlikely to have interfered with our results. The higher yield from the pooled specimens also indicated that toxicity to the cell layer was not increased by pooling the urethral and cervical swabs.

Paavonen isolated *C trachomatis* from the urethra only in 25% of 99 women who were consorts of men with non-gonococcal urethritis.<sup>2</sup> This higher urethral isolation rate was explained by Bradley *et al* as probably being due to the relatively lower rate of isolation from the cervix (75%) found by Paavonen.<sup>7</sup> Table II shows that other workers (except Johannisson *et al*<sup>10</sup>) isolated *C trachomatis* from the urethra alone in only 1-8% of women.<sup>2 7 8 10-12</sup> Though Bump and Copeland failed to isolate *C trachomatis* from the urethra of 86 women with chronic urological complaints,<sup>13</sup> Stamm *et al* showed the possible role of this organism in the aetiology of "urethral syndrome" in women. Nevertheless, some workers believe that the

TABLE II No (%) of patients yielding *Chlamydia trachomatis* from urethra, cervix, or both

Publications	Urethra only	Cervix only	Both	Total
Dunlop <i>et al</i> <sup>8*</sup>	6 (9)	43 (61)	22 (31)	71
Dunlop <i>et al</i> <sup>8†</sup>	1 (1)	79 (71)	31 (28)	111
Bradley <i>et al</i> <sup>7</sup>	5 (4)	44 (38)	66 (57)	115
Moller <i>et al</i> <sup>11</sup>	2 (5)	15 (41)	20 (54)	37
Johannisson <i>et al</i> <sup>10</sup>	19 (15)	75 (61)	29 (24)	123
Paavonen <sup>2</sup>	25 (25)	28 (28)	46 (47)	99
Paavonen <i>et al</i> <sup>12</sup>	2 (7)	13 (48)	12 (44)	27
Present study‡	11 (11)	22 (22)	67 (67)	100

\* Results of first culture, excluding that of a patient yielding chlamydiae from rectum only.

† Results of three consecutive cultures, excluding those of a patient yielding chlamydiae from rectum only.

‡ Excluding one patient yielding chlamydiae from the pooled specimen only.

urethra is contaminated secondary to cervical infection and suggest that there is no indication to take urethral swabs from women. We have shown the advantages of the urethral swab, however, in terms of increased yield and cost benefit, if it is incorporated in a pooled specimen.

In conclusion, the pooling method has worked well in our hands, but we feel that any diagnostic laboratory proposing to adopt it should not do so without validating it in their own population. Furthermore, interval testing not only provides information about the reproducibility of a method, but may be necessary to detect incubating infection. It is our intention to assess further the sensitivity of our diagnostic service in this way.

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