Toxic effect of sampling swabs and transportation test tubes on the formation of intracytoplasmic inclusions of *Chlamydia trachomatis* in McCoy cell cultures

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SUMMARY The ability of *Chlamydia trachomatis*, immunotype E, to produce intracytoplasmic inclusions in cycloheximide-treated McCoy cells after being exposed to different types of sampling swabs in experimentally infected transport medium was studied. A larger number of inclusions was obtained with cotton-tipped aluminium and rayon-tipped plastic swabs than with calcium-alginate-tipped aluminium and cotton-tipped wooden swabs ($P < 0.0001$). Transport medium stored in glass tubes caused a cytopathic effect when inoculated on to McCoy cell cultures; no such effect occurred with plastic tubes. When cotton-tipped aluminium instead of calcium-alginate-tipped aluminium swabs were used to collect 50 male urethral specimens significantly more were chlamydia-positive ($P < 0.025$). This was also true when cotton-tipped aluminium swabs were used instead of alginate-tipped swabs in a study of 123 cervical specimens ($P < 0.01$). When the calcium-alginate-tipped aluminium and cotton-tipped wooden swabs were shaken in the transport medium after sampling from the male urethra and the cervix, instead of being left in the medium during transport to the laboratory, more specimens were chlamydia-positive and a greater number of chlamydial inclusions were found per culture-positive sample; these results were, however, not statistically significant ($P > 0.05$).

Introduction

Specimens for the diagnosis of infections with *Chlamydia trachomatis* are usually collected with cotton-tipped swabs. Sticks of wood or metal, including aluminium, tipped with pure cotton or calcium-alginate cotton have been recommended. The swabs are generally transported in sucrose-phosphate buffer (2-SP),¹ containing fetal calf serum or albumin and various antimicrobial agents. Both glass and plastic test tubes are used for transporting specimens.

For the isolation of *C. trachomatis*, specimens are inoculated into cell cultures; McCoy cells are now the most widely used cell line. To render the tissue cells highly susceptible to infection by chlamydia different pretreatments, including irradiation and cytostatic agents, are used. Comparative studies have shown

that treatment with cycloheximide² is the most efficient.³

This study was carried out to determine the influence on the formation of intracytoplasmic chlamydial inclusions in cycloheximide-treated McCoy cell cultures after exposure of the organisms in transport medium to different commercially available sampling swabs and their sticks and cotton components. Comparative studies on urethral and cervical specimens showed great variations in the frequency of isolation of chlamydia with various types of sampling swabs and transportation test tubes.

Material and methods

SAMPLING SWABS AND TUBES

The following sampling swabs were tested: Calgiswab Nos I, II, and IV (Inolex Corp, Glenwood, Ill, USA); Torrent's Sterile Applicators (Torrent Corp, Lake Geneva, Wis, USA); Pur-Wraps cotton-tipped swab (Hardwood Products Co, Gilford, Mass, USA); ENT Sterile Applicator No 1054 (Medical Wire &

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Equipment Co Ltd, Corsham, Wilts, England); Nunc's charcoal-impregnated cotton-tipped swab (Nunc, Roskilde, Denmark); and Culturette (Marion Scientific Corp, Kansas City, Miss, USA). Only the swabs from the ENT and Culturette kits were used. Glass (Labora AB, Stockholm, Sweden) and plastic (Nunc) test tubes were used.

TRANSPORT MEDIUM

C. trachomatis, immunotype E, was suspended in sucrose-buffer (2-SP),¹ containing 10% fetal calf serum (Flow Laboratories Ltd, England), 20 µg gentamicin (Schering), 5 µg amphotericin B (Squibb), and 100 µg vancomycin (Eli Lilly) per ml. The final concentration of chlamydia corresponded to approximately 0.8×10^2 inclusion-forming units (ifu) per ml 2-SP or tissue culture medium (see below).

ISOLATION TECHNIQUE

Cultures for *C. trachomatis* were performed on cycloheximide-treated McCoy cells²; RPMI 1640 (Flow Laboratories) was used as the tissue culture medium. Volumes of 0.2 ml of the specimens were inoculated on to the cell cultures containing 1 ml tissue culture medium. The cells were stained with iodine after incubation at 37°C for 72 hours and the number of chlamydial ifu studied. The cultures were performed in flat-bottomed plastic vials (Luckham, London).

FORMATION OF CHLAMYDIAL INCLUSIONS IN CELL CULTURES

Influence of sampling swabs

One millilitre of the suspension of chlamydia (see above) was added to plastic tubes, which were stored at 4°C for two days before the suspensions were cultured. In similar experiments the different sampling swabs were placed in the infected 2-SP.

In addition, cotton and pieces of sticks from the different types of sampling swabs were placed in chlamydia-infected McCoy cell cultures. The cultures were performed as described above.

CYTOPATHIC EFFECT ON MCCOY CELLS BY TRANSPORT MEDIUM

Volumes of 0.5 ml 2-SP transport medium,¹ which had and had not been stored in glass and plastic tubes for two days, were dispersed in McCoy cell cultures containing 0.5 ml fresh tissue culture medium. The cultures were incubated for three days and studied for cytopathic effects (CPE).

DETERMINATION OF COPPER AND ZINC IN WASHING FLUID

The contents of copper and zinc in the fluid used to wash the glass and plastic tubes were determined by flame ionisation spectrometry.⁴

CHLAMYDIAL ISOLATION FROM SPECIMENS USING DIFFERENT SAMPLING SWABS

Urethral specimens were collected from 50 male STD clinic patients using calcium-alginate (Calgiswab No I) and cotton-tipped (ENT Sterile Applicator) aluminium swabs. Cervical samples were collected from 123 female patients using cotton-tipped wooden (Pur-Wraps) and cotton-tipped aluminium (ENT Sterile Applicator) swabs. The order in which the swabs were used was alternated in successive patients. The specimens were transported in plastic tubes and cultured as described above. The transport time did not exceed six hours.

In one study two sets of urethral (from 52 male STD clinic patients) and cervical (from 26 gynaecological outpatients) specimens were collected with Calgiswab No I and ENT Sterile Applicators respectively. One swab was vigorously shaken in the transport medium immediately after sampling and the swab was discarded. The other was left in the medium during transport to the laboratory. The results of the chlamydial cultures from these sets of specimens were compared.

CHLAMYDIAL ISOLATION FROM SPECIMENS USING GLASS AND PLASTIC TUBES FOR TRANSPORTATION

The culture results for chlamydial isolation from cervical and urethral specimens from 42 STD clinic patients transported in glass and plastic tubes were compared. The urethral specimens were collected with Calgiswab No I and the cervical specimens with Torrent's Sterile Applicators. The transport medium and the chlamydial culture technique were those already described.

STATISTICAL METHODS

Sign and Kruskal-Wallis tests were used.

Results

FORMATION OF CHLAMYDIAL INCLUSIONS FROM EXPERIMENTALLY INFECTED SAMPLES

The percentage of ifu in McCoy cell cultures inoculated with the experimentally infected transport medium, in which different sampling swabs had been placed for 72 hours, was compared with the number of ifu obtained in cultures of aliquots of the same specimens which had not been exposed to any swabs (table I). The cotton-tipped aluminium and rayon-tipped plastic swabs (ENT) were less toxic than the other swabs tested ($P < 0.001$).

The percentage of ifu in McCoy cell cultures made in the presence of sticks and cotton from the various swabs was compared with unexposed control cultures (table II). Calcium-alginate cotton gave fewer

TABLE I *Percentage of inclusions of Chlamydia trachomatis, immunotype E, in cycloheximide-treated McCoy cell cultures inoculated with specimens stored in plastic tubes for 48 hours in the presence of various sampling swabs compared with controls*

Sampling swab			% of chlamydial inclusions compared with control cultures
Stick	Tipped with	Trade name*	
Aluminium	Calcium-alginate	Calgiswab No I	13
Wood	Calcium-alginate	Calgiswab No II	0
Aluminium	Calcium-alginate	Calgiswab No IV	8
Aluminium	Cotton	ENT	64
Wood	Cotton	(Torrent)‡	8
Wood	Cotton	Pur-Wraps	4
Plastic	Rayon	Culturette	59
Wood	Carbon-impregnated cotton	(Nunc)‡	†

*For further information, see text

†Cell cultures unreadable because of carbon particles

‡Name of manufacturer

chlamydial inclusions than pure cotton. Differences in the toxic effect of wooden sticks from various manufacturers were also demonstrated. The use of charcoal-impregnated cotton resulted in unreadable cultures owing to the presence of carbon particles.

COPPER AND ZINC IN WASHING FLUID

The washing solution from glass tubes tested by flame ionisation spectrometry contained 1.8 mg zinc and 2 mg copper per litre; the corresponding figures from the plastic tubes were 1.3 and 1.8 respectively.

CHLAMYDIAL CULTURE RESULTS AFTER TRANSPORTATION OF SPECIMENS IN GLASS AND PLASTIC TUBES

Studies in which urethral specimens (collected with Calgiswab No I) and cervical specimens (collected with Torrent's Sterile Applicators) from the same 42 patients were transported in both glass and plastic tubes showed that the latter tubes gave more positive isolates. Thus of the six chlamydia-positive

specimens transported in plastic tubes, only four gave positive results when glass tubes were used. In no instance was the reverse found. A discrepancy in the number of ifu per coverslip in favour of the plastic tubes was also found. Thus, in the cultures of the four specimens giving positive results after transportation in both glass and plastic tubes, 207 more inclusions were found when the specimens were sent to the laboratory in plastic tubes. However, none of these differences was statistically significant.

CHLAMYDIAL ISOLATION RATE WITH VARIOUS SAMPLING AND SPECIMEN TRANSPORTATION METHODS

Aluminium swabs tipped with cotton gave more chlamydial isolates from male patients than calcium-alginate-tipped aluminium swabs ($P < 0.025$). Similarly in women, sampling with this former type of swab gave a higher chlamydial isolation rate than with cotton-tipped wooden swabs ($P < 0.01$) (table III).

When the swabs from urethral and cervical specimens were shaken instead of being left in transport medium, the number of chlamydia-positive specimens as well as of ifu was increased, but the differences were not statistically significant (table IV).

Discussion

With the experimental conditions used in this study, we were unable to establish whether the formation of intracytoplasmic chlamydial inclusions in McCoy cell cultures by the different sampling swabs used, and by sticks and cotton from these swabs, was inhibited owing to an effect on the chlamydia or on the McCoy cells or on both. Nor could we determine whether the pinocytosis of chlamydia was affected by alterations of the McCoy cell membrane or changes of the "milieu interieur" of the host cells or by both.

TABLE II *Percentage of inclusions of Chlamydia trachomatis, immunotype E, in cycloheximide-treated McCoy cell cultures performed in the presence of cotton and sticks from sampling swabs compared with controls*

Sampling swab	Trade name*	% of chlamydial inclusions compared with control cultures
Stick		
Aluminium	Calgiswab Nos I and IV	67
Aluminium	ENT	74
Wood	Calgiswab No II	47
	(Torrent)†	74
	Pur-Wraps	83
Cotton		
"Pure" cotton	(Medical Wire)†	63
	(Torrent)†	27
	Pur-Wraps	25
Calcium-alginate cotton	Calgiswab Nos I, II, and IV	0.2

*For further information, see text

†Name of manufacturer

TABLE III Comparison of the frequency of chlamydial isolation in male and female STD clinic patients using different sampling swabs

Patients	Sampling site	Sampling swab			No of specimens	Chlamydia-positive	
		Stick	Tipped with	Trade name*		No	%
Men	Urethra	Aluminium	Calcium-alginate	Calgiswab I	50	7	14
		Aluminium	Cotton	ENT	50	14	28
Women	Cervix	Wood	Cotton	(Torrent)†	123	3	2.4
		Aluminium	Cotton	ENT	123	11	8.9

*For further information, see text

†Name of manufacturer

However, of the different sticks and cotton from the swabs used, only calcium-alginate caused a CPE of McCoy cells when cultured in its presence for 48 hours.

Secretion from the male accessory genital glands is rich in metal ions, such as zinc.⁵ This is also true of urethral secretion, which contains up to 20% of fluid from these glands. In a previous study⁴ we showed that the presence of seminal fluid and some of its components had a negative influence on the formation of intracytoplasmic chlamydial inclusions. We also found that seminal fluid experimentally infected with chlamydia and stored in glass vials before culture gave fewer inclusions than when plastic tubes were used. Components of seminal fluid, including copper and zinc, had a negative effect on the formation of chlamydial inclusions. In this earlier study⁴ we also found that the addition of EDTA to seminal fluid experimentally infected with chlamydia resulted in a larger number of inclusions in subsequent McCoy cell cultures compared with similar experiments without this acid.

The washing fluid from glass tubes contained several metals—for example, copper and zinc—in concentrations as high as those found in secretion from the male accessory genital glands. The concentrations of these metals were lower in the washing fluid from the plastic tubes. Whether this difference might have contributed to the finding that plastic was less toxic than glass, however, is not known. Cations may change the characteristics of cell membranes⁶ and thereby also interfere with the pinocytosis of chlamydia. One other possibility not yet explored is

that chlamydia may stick more readily to glass than to plastic surfaces, resulting in fewer inclusions in subsequent cultures of the washing fluid.

The negative effect of calcium alginate on the formation of intracytoplasmic chlamydial inclusions in McCoy cell cultures might, at least partly, be due to their contribution of calcium ions to the cultures. In an earlier attempt⁴ to determine the influence of calcium ions on the number of ifu in McCoy cell cultures, a film of precipitated material made reading of the cells impossible.

It has been suggested that the toxic effect of wooden sampling sticks on certain bacteria, such as gonococci, may be partly explained by their content of fatty acids. This might also apply to chlamydia.

When explaining the difference in the culture results of tests using different swabs and the difference between tests in which the swab was shaken or left in the tube during transportation, the possibility that chlamydia differ in their attachment to various types of cotton must also be taken into account. We do not recommend that the sampling swab is shaken and thereafter discharged as a routine procedure in the cultural diagnosis of genital chlamydial infections, because when the shaking is not vigorous enough false-negative results may occur.

Apart from being non-toxic, a swab for collecting specimens for culture of genital chlamydia should have a calibre that allows it to be introduced approximately 3-4 cm into the urethra without causing pain. This seems to be a reason why Calgiswabs Nos I and IV are widely used. The swab which we found to be

TABLE IV Comparison of the frequency of chlamydial isolation and inclusion count from the urethra of men attending an STD clinic and from the cervix of women attending a gynaecological outpatient clinic when the sampling swab was either left in the transport tube or removed after being shaken in the tube

Patients	Sampling site	No of patients	Sampling swab in transport tube	Chlamydia-positive		No of chlamydial inclusions ($\times 10^3$)
				No	%	
Men	Urethra	134	Left	26	19	3.7
			Shaken	30	22	5.8
Women	Cervix	67	Left	8	12	3.3
			Shaken	10	15	8.4

least toxic (together with the Culturette) was the aluminium cotton-tipped swab (ENT No 1054); but this is too thick to be ideal for urethral sampling. The same company (Medical Wire & Equipment Co Ltd) produces a similar nasopharyngeal swab for use in children. This is, however, too thin to be ideal for urethral or cervical sampling.

The reason for using carbon-impregnated swabs for collecting specimens for the culture of *Neisseria gonorrhoeae* is that carbon particles adsorb substances toxic to gonococci that may be released from the cotton swab, the stick, the medium, and the clinical specimen itself. The presence of carbon particles, however, interferes with the reading of cell cultures and thus makes these swabs unsuitable for chlamydial culture studies.

The results of this study stress the importance of testing swabs and tubes for use in the collection and transportation of specimens for the cultural diagnosis of *C. trachomatis* before their use is generally recommended.

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