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Effect of Swab Type and Storage Temperature on the Isolation of *Chlamydia trachomatis* from Clinical Specimens

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We evaluated various swabs for the recovery of *Chlamydia trachomatis* and found that only 7 of 14 swabs tested resulted in a greater than 50% recovery of organisms. Storage of chlamydial specimens at 4°C for more than 1 day resulted in a rapid loss of viability, whereas freezing of specimens at −70°C resulted in only a marginal decrease in the isolation rate.

For the past two decades, the laboratory diagnosis of *Chlamydia trachomatis* has been based on the isolation of this organism in cell cultures. The success of laboratories in culturing chlamydiae is directly related to the collection of an appropriate specimen by the physician and the preservation of chlamydiae in the specimen before arrival in the laboratory. Since most microbiology laboratories do not have expertise in cell culturing techniques, regional laboratories are usually called upon to culture chlamydiae. Regionalization necessitates the transportation of specimens to the laboratory, usually from substantial distances, bringing about a delay in specimen inoculation anywhere from overnight to as long as 3 days over a weekend. Recent reports have shown that a delay of only 24 h results in a significant decrease in the isolation rate for chlamydiae (8, 10). Probably more important than the delay in inoculating specimens is the type of swab used for collecting the specimens, as some types of swabs have recently been shown to decrease the isolation rate (4, 9). Despite continuing efforts to encourage the use of nontoxic swabs, most laboratories still receive specimens collected with inappropriate swabs. At the request of several smaller laboratories with less expertise, we evaluated 14 types of swabs for their effect on the viability of *C. trachomatis* and reassessed storage conditions for holding clinical specimens.

Swabs were evaluated by inoculating with one swab a tube containing 1 ml of transport medium and a known number of inclusion-forming units of *C. trachomatis* serovar L2 LGV 434 overnight at 4°C to simulate clinical specimens. On the next day, a sample of the medium was inoculated in triplicate onto McCoy cell monolayers in microculture plates (1), and inclusions were stained with iodine at 48 h as described previously (3). At least four swabs of each type were tested on different days, and inclusion counts for duplicates varied minimally. The results were averaged and expressed as a percentage of control results (counts with no swab).

The recovery of *C. trachomatis* varied markedly depending upon the type of swab (Table 1). The best recovery rates were experienced with cotton on aluminum and resulted in a decrease in inclusion counts of only 0 to 13% from the counts for the control tubes containing no swabs. The recovery of chlamydiae from 4 different cotton-on-plastic swabs ranged from 23 to 83%. Two calcium alginate swabs were toxic to cell cultures, resulting in no visible inclusions. The remaining calcium alginate swab yielded a 77% recovery of *C. trachomatis*. Two of three dacron-on-plastic swabs were toxic to the cells, and the third yielded a recovery of 81%. The single cotton-on-wood swab tested appeared to be inhibitory to chlamydiae and decreased the inclusion count to an undetectable level. We did not observe significant differences between batches of swabs from the same manufacturer.

To investigate the effect of storing specimens at different temperatures, we collected urethral and endocervical specimens from patients attending a sexually transmitted disease clinic at the Hamilton General Hospital. Specimens were collected with cotton-tipped swabs (ENT or Pernasal; Medical Wire & Equipment Co., Corsham, Wiltshire, United Kingdom) and placed into a tube containing two glass beads and 1.5 ml of sterile transport medium consisting of Eagle minimal essential medium, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 5.4 mM sodium bicarbonate, 10 μg of gentamicin per ml, and 1% bovine serum albumin (fraction V; Sigma Chemical Company, St. Louis, Mo.). Specimens were transported to the laboratory on the same day on wet ice, mixed for 30 s, and divided into four tubes; the tubes were inoculated immediately; held at 4, 23, or −70°C; or snap-frozen in a dry ice-acetone bath. The isolation of chlamydiae was performed by the microculture technique with preformed monolayers of McCoy cells in 96-well plates as described previously (1). Plates were incubated at 37°C and stained with iodine after 48 h. Only positive specimens (those yielding chlamydiae after immediate inoculation) were used in the analysis of the effect of storage at different temperatures.

Compared with immediate inoculation, the best recovery occurred after the specimens were held at 4°C for 24 h (Table 2). Slow freezing or snap-freezing provided good recoveries. Of the 36 positive specimens, 31 (86.1%) yielded chlamydiae after being snap-frozen in dry ice-acetone, and 33 (91.7%) yielded chlamydiae after being slowly frozen to −70°C. Since each specimen could not be used for all storage times and temperatures, different numbers of specimens were evaluated at different storage conditions (as indicated by the denominators in Table 2). The recovery of chlamydiae from positive specimens stored at 4°C decreased quickly, with only one specimen yielding chlamydiae after 7 days. None of the three specimens stored for 8 days at 4°C yielded chlamydiae. Storage at 23°C resulted in a rapid decrease in the isolation rate from 34.4% at 1 day to 0% at 2 days.

Our results show that a number of commercially available swabs, particularly those containing calcium alginate or

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TABLE 1. Recovery of *C. trachomatis* LGV 434 from various swabs

Swab	Swab type	% Inclusion count[a]
Pernasal	Cotton and aluminum	100
ENT	Cotton and aluminum	96.5
STD EZE (males) (Medical Media Laboratory, Boring, Oreg.)	Cotton and aluminum	66.8
American Scientific Products, McGaw Park, Ill.	Cotton and plastic	82.9
STD EZE (females) (Medical Media Laboratory)	Cotton and plastic	50.5
Tomac, Div. McGaw Supply Ltd., Mississauga, Ontario, Canada	Cotton and plastic	29.6
NCS (NCS Diagnostics Inc., Mississauga, Ontario, Canada)	Cotton and plastic	22.6
Purr-Wrapps (Hardwood Products Co., Guilford, Maine)	Calcium-alignate and aluminum	77.3
Spectrum (Spectrum Diagnostics, Glenwood, Ill.)	Calcium-alignate and aluminum	Toxic[b]
Inolex Calgiswab Type II (American Can Co., Glenwood, Ill.)	Calcium-alignate and aluminum	Toxic
American Scientific Products	Dacron and plastic	80.8
Microtrak	Dacron and plastic	Toxic
Spectrum	Dacron and plastic	Toxic
Spectrum	Dacron and plastic	Toxic
Tomac	Cotton and wood	0

[a] Average inclusion count for four to six swabs incubated overnight with approximately 100 inclusion-forming units of *C. trachomatis* LGV 434 and inoculated in triplicate in microculture plates. Results are expressed as a percentage of control counts (no swab).

[b] Some swabs were toxic to McCoy cells and destroyed the monolayers, precluding a determination of chlamydial inclusions.

wood, may be unsuitable for the collection of chlamydial specimens. Only 7 of 14 available swabs tested demonstrated a better than 50% recovery of *C. trachomatis* LGV 434 after overnight incubation at 4°C. The effect of swab materials on the viability of trachoma biotype strains or urogenital isolates is unknown and may be greater than that observed for LGV biotype strains. The variability we observed for swabs made of similar materials may be attributed to differences in how tightly the swab fibers are bound to the shaft. This hypothesis was not assessed in this study, and the role of binding in the recovery of chlamydiae from swabs remains obscure. Smith and Weed recently demonstrated that a commercial swab-transport system (Culturette) yielded 40% fewer positive specimens than laboratory-prepared sucrose phosphate transport medium (9). These authors also showed that both calcium alginate and rayon swabs may bind chlamydial elementary bodies irreversibly and decrease inclusion counts. They did not determine, however, if cotton swabs also bind chlamydial elementary bodies. Mardh and Zeeberg reported that cotton swabs were superior to calcium alginate swabs and that plastic or aluminum shafts were superior to wood shafts (4). Since calcium alginate has also been shown to be toxic for herpes simplex virus (2) and since wooden swabs are toxic for *Ureaplasma urealyticum* and *Neisseria gonorrhoeae* (6), it would be prudent not to use these materials for optimal isolation of these sexually transmitted organisms. Instead, cotton, rayon, or dacron tips on plastic or aluminum shafts should be used. In addition, vigorous mixing of the transport tube containing the collection swab and two glass beads (to release chlamydial elementary particles from swab fibers) followed by removal of the swab from the tube may help to minimize the trapping of organisms in swabs during overnight storage.

In some settings in which specimens are transported to regional laboratories, it is impossible to inoculate specimens on the same day that they are collected. In the present study, we found that freezing specimens at −70°C or storing them overnight at 4°C for 1 day resulted in a minimal loss of positive results (8 and 4%, respectively [Table 2]); however, storage at 4°C for longer than 1 day significantly decreased the number of positive isolations. Reports from Europe have shown that storage at −70°C results in an 11 to 20% decrease in the isolation rate and that storage at 4°C for 24 to 48 h yields 11 to 24% fewer positive specimens (5, 7, 8, 10). In contrast, however, freezing specimens in liquid nitrogen did not reduce the isolation rate in one study (5). Tjiam et al. reported that the addition of a cryoprotective agent (sucrose) to the transport medium improved the recovery of chlamydiae (10). Ngeow et al. noted, as we have in the present study, a correlation between the number of inclusion-forming units in the original specimen and the loss of culture-positive results after storage, with low inclusion counts increasing the risk of losing positive isolates (5). Tjiam et al. also reported that up to 4% of the specimens which were negative upon first inoculation were positive after being frozen or stored at 4°C for 48 h (10); this phenomenon may be due to the intracellular localization of chlamydiae and the lysis of host cells during freezing or storage at 4°C or to the disintegration of toxic material during storage. We did not experience this phenomenon in our limited studies. The initial infectivity (titer) of the specimen and perhaps bacterial contamination would be important factors for the successful reisolation of chlamydiae from frozen specimens; these factors were not reflected in our study.

Various laboratories are in the process of evaluating the newly developed nonculturing techniques for detecting *C. trachomatis*. Both the Chlamydiazyme (Abbott Laboratories, North Chicago, Ill.) and Microtrak (Syva Diagnostics, Seattle, Wash.) tests have swabs included for use with their products. As more laboratories begin using these tests, they

TABLE 2. Effect of storage at different temperatures on the viability of *C. trachomatis* in clinical specimens

Temp (°C)	No. of specimens positive/no. of specimens tested (%) on indicated day of storage							
	1	2	3	4	5	6	7	8
4	22/23 (95.7)	15/27 (55.6)	22/39 (56.4)	10/27 (37)	6/29 (20.7)	8/31 (25.8)	1/7 (14.3)	0/3 (0)
23	10/29 (34.4)	0/29 (0)	0/29 (0)					
−70 (slow-freezing)			33/36 (91.7)					
−70 (snap-freezing)			31/36 (86.1)					

may choose to evaluate them by comparing each to cell culturing for chlamydial isolation. We found that the Microtrak swab was toxic to McCoy cell cultures and that a single swab could not be used to compare direct immunofluorescence staining to culturing. If laboratories are performing comparisons, two specimens, one taken with a nontoxic swab (for culturing), will have to be collected. The swabs provided with the Chlamydiazyme test (STD EZE swabs for both males and females) yielded a reasonable recovery of chlamydiae and could, therefore, be used for either the Chlamydiazyme test or cell culturing.

Our results emphasize the need for freezing or refrigerating specimens and for rapidly transporting them to the laboratory to ensure the successful isolation of *C. trachomatis*. If specimens must be held longer than 1 day, they should be frozen until they can be inoculated. The poor recovery of chlamydiae from some swabs, in particular, calcium alginate swabs or wooden-shafted swabs, indicates that laboratories isolating chlamydiae should periodically evaluate the commercially available swabs used in their institutions.

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