Effect of Storage Temperature on Survival of *Chlamydia trachomatis* after Lyophilization

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Lyophilized preparations of *Chlamydia trachomatis* were made to investigate how well they would survive storage at four relevant incubation temperatures for 1 week and 1 month. Good viability was maintained by storage at either 4°C or 20°C for 1 week. If the ambient temperature is not too high, short-term transportation of *C. trachomatis* is achievable through lyophilization.

*Chlamydia trachomatis* is an important cause of both trachoma and sexually transmitted infections (6). In the United Kingdom especially, genital infections caused by *C. trachomatis* have risen dramatically in the last few years (3). Although nucleic acid amplification testing is now becoming more common for laboratory diagnosis, growth of *C. trachomatis* is still required, for example, in pathogenicity studies. Difficulties can arise in trying to transport *C. trachomatis* for growth-related studies, as the ATCC recommendation is that cultures must be kept frozen. In some parts of the world, this recommendation would not pose any problems; however, significant time delays do exist in other areas, such as Africa, and a concern is that the transported cultures may no longer remain frozen and therefore become nonviable.

Of course, a common method for preservation and storage of microorganisms is via lyophilization. This method can be applied to most bacteria, and in this state, transportation does not require freezing. Unfortunately, there is little published work on lyophilization of *C. trachomatis*, and although it has been successful, preservation was attempted only by keeping lyophilized *C. trachomatis* LGV2 at 4°C (7). We wished to explore whether lyophilized *C. trachomatis* could remain viable at temperatures more closely mimicking those of ambient transportation and without artificial cooling.

Two *C. trachomatis* strains were tested, a fresh clinical strain (F) which was identified by genotyping and a known LGV2 strain which was confirmed by genotyping (4). Elementary body (EB) suspensions were prepared by 48 h of growth at 37°C in 5% CO₂ and were then harvested and purified as described by Caldwell et al. (2). The growth medium was Eagle’s minimal essential medium (Cambrex Biosciences, Wokingham, United Kingdom) plus 10% fetal calf serum (FCS), 2 μg/ml cycloheximide, and antibiotics. The EB pellet was resuspended in the suspension medium (FCS plus 7.5% glucose buffer), as described by Theunissen et al. (7). Determination of viable EBs was made by preparing serial 10-fold dilutions in growth medium and then transferring 1 ml to Trac bottles (Fisher Scientific, Loughborough, United Kingdom) containing coverslips. These were incubated for 48 h, and the coverslips were fixed in methanol and stained with fluorescein-conjugated monoclonal antibody specific for *C. trachomatis* (Dako, Ely, United Kingdom). The number of inclusion bodies in 20 fields was counted, and the average was taken to give the mean number of infection-forming units.

For lyophilization, EB pellets of a known number of infection-forming units, typically around 3 × 10⁶ to 4 × 10⁶, suspended in FCS plus 7.5% glucose buffer, were pipetted in 100-μl volumes into five sterile freeze-dry ampoules fitted with light plugs of nonabsorbent cotton wool. Ampoules were placed in an Edwards Modulyo EF4 lyophilizer (Crawley, United Kingdom) that had been precooled and underwent the conventional two-stage lyophilization process. A vacuum was drawn, and the ampoules were centrifuged until frozen and then kept in the lyophilizer overnight. On the following day, the ampoules were removed from the lyophilizer, the cotton plugs were pushed halfway down the tubes, and the tubes were constricted by being heated. The ampoules were replaced on the tube adaptor of the lyophilizer, and a vacuum was drawn and maintained for several hours. After this time, the tubes were sealed under vacuum and were ready for storage.

Recovery was determined at 1 week and 1 month after incubation at 4°C, 20°C, 30°C, or 37°C. The ampoules were broken as recommended by the ATCC (1). Sterile distilled water (100 μl) was added to each ampoule, and after the contents had been mixed, 10-fold dilutions were made in growth medium. One milliliter was added to each ampoule, and after the contents had been mixed, 10-fold dilutions were made in growth medium. One milliliter was added to each ampoule, and after the contents had been mixed, 10-fold dilutions were made in growth medium.

Our data clearly show (Table 1) that good viability of both serovars is maintained by storage at 4°C or 20°C for 1 week, with percent recovery rates between 0.29% ± 0.22% and 0.69% ± 0.53%. In general, viability declined markedly after 1 month of storage at both 4°C and 20°C, with percent recovery rates ranging from 0.043% ± 0.011% to 0.53% ± 0.83%. At 30°C, viability after 1 week was much reduced (0.021% ± 0.010% to 0.048% ± 0.022%), although incubation for 1 month, surprisingly, did not reduce viability (0.021% ± 0.014% to 0.041% ± 0.014%) too much more. There was no recovery at 37°C, even after only 1 week of incubation. Similar to previous findings by Theunissen et al. (7), we also found in a
separate experiment that changing the suspension medium to sucrose phosphate glutamine buffer resulted in decreased viability (data not shown).

To confirm our above findings on the effect that storage temperature had on viability of \textit{C. trachomatis} following lyophilization, we also reconstituted a separate ampoule which had been stored for 1 week and inoculated the contents into a 25-cm flask of McCoy cells. This experiment was performed in triplicate. After 72 h, the infected cells were scraped, concentrated, and passaged onto a fresh cell sheet. After a further 72-h incubation, inclusions were observed by light microscopy and the percentage of infected McCoy cells was estimated. We confirmed that good viability (30 to 90\%) was possible after storage at 4°C or 20°C but that recovery after storage at 30°C or 37°C was poor (5 to 10\%) or unsuccessful, respectively.

It was shown a long time ago that a cold storage temperature is important for maintaining viability of \textit{C. trachomatis} (5). This explains why usual transportation of \textit{C. trachomatis} is on ice, with its associated difficulties if there are delays and/or a high ambient temperature. We have shown that with FCS plus 7.5\% glucose as a suspension medium, reasonable viability of \textit{C. trachomatis}, like that of other bacteria, can be achieved if a large enough inoculum is used initially, and we have demonstrated that recovery of serovar E is equal to or slightly better than that of LGV2. We have also demonstrated that recovery of \textit{C. trachomatis} after storage at 20°C is comparable to that after storage at 4°C and that even after storage at 30°C for 1 month, some viability is still maintained. However, the recovery levels reported here (below 1\%) may not be good enough to preserve low levels of EBs, which can be isolated routinely during clinical sampling. Nevertheless, lyophilization can now be used as a useful alternative to freezing of large inocula of \textit{C. trachomatis} for maintaining viability during transportation, where ambient temperatures do not rise above 20°C, preferably within a 1-week period. If transportation was known to be difficult and/or prolonged and unsuitable for shipping frozen cultures, it might also be an advantage to send lyophilized ampoules on ice, as cooler ampoule temperatures will improve overall recovery.

\section*{REFERENCES}

\begin{table}[h]
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\begin{tabular}{l|c|c|c}
\hline
\textbf{Serovar} & \textbf{Storage temp (°C)} & \textbf{1 wk} & \textbf{1 mo} \\
\hline
E & 4 & 0.52 ± 0.38 & 0.53 ± 0.83 \\
 & 20 & 0.69 ± 0.53 & 0.42 ± 0.71 \\
 & 30 & 0.048 ± 0.022 & 0.041 ± 0.014 \\
 & 37 & — & — \\
LGV2 & 4 & 0.59 ± 0.43 & 0.061 ± 0.037 \\
 & 20 & 0.29 ± 0.22 & 0.043 ± 0.011 \\
 & 30 & 0.021 ± 0.010 & 0.021 ± 0.014 \\
 & 37 & — & — \\
\hline
\end{tabular}
\caption{Effect of temperature and time on recovery of lyophilized \textit{C. trachomatis} after storage at 1 week and 1 month.}
\end{table}

\textsuperscript{a} Values are means ± standard deviations.
\textsuperscript{b} —, no recovery.