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# Long-Term Preservation of *Bordetella pertussis*

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Viability of *Bordetella pertussis* was preserved when glycerol broth suspensions were quick frozen and stored at −70 C for as long as 45 months.

In laboratories that are not equipped with lyophilization facilities, periodic subculturing of fragile microorganisms such as *Bordetella pertussis* is required to preserve original isolates. This is not only time-consuming and expensive but may increase the loss of the microorganisms through contamination or result in the appearance of variant forms. The method of preservation reported here for *B. pertussis* uses a glycerol suspension first described by Hollander and Nell (1) and later extended by Howard (2) and follows the freezing principles summarized by Weiser and Osterud (3).

Seventeen of the *B. pertussis* strains in this study were isolated from patients receiving care for whooping cough at the Los Angeles County-University of Southern California Medical Center from July to December 1967. A lyophilized culture of ATCC (American Type Culture Collection) 9340 was also subcultured and stored. Pure cultures of *B. pertussis* were grown on Bordet-Gengou (B-G) media (Difco) containing 25% fresh, sterile defibrinized sheep blood. After incubation at 36 C for 75 hr in a humid chamber, the cultures were overlaid with 10 ml of *Brucella* broth (Albimi Laboratories, Flushing, N.Y.) containing 15% glycerol. By gentle agitation with a flattened glass rod, the colonies were dislodged from the B-G agar and dispersed into the broth medium. The resultant bacterial suspensions were transferred to 15-ml screw-capped tubes for further vigorous agitation. Suspensions were distributed in 0.5-ml portions into 2-dr screw-capped vials (Kimble Products, Toledo, Ohio) that were held in a precooled (−70 C) aluminum block designed with wells 17 mm in diameter to accommodate the vials. Bacterial suspensions solidified in the vials instantaneously. Vials were then removed and stored at −70 C. Periods of storage at which viability checks of frozen *B. pertussis* suspensions were made are shown in Table 1. All recultured isolates retained their smooth colony appearance and morphology (Gram stain). Identification was confirmed by a positive agglutination reaction to *Bordetella* monospecific factor 1 antiserum (Burroughs Wellcome and Co., Tuckahoe, N.Y.).

It is apparent that quick freezing and subsequent storage of glycerol broth suspensions of *B. pertussis* at −70 C are highly effective in maintaining the viability of *B. pertussis* for periods as long as 45 months. The ease with which the method can be performed can be an asset in laboratories without lyophilization equipment for the preservation of *B. pertussis* and other microorganisms.

TABLE 1. *Viability of frozen (−70 C) B. pertussis suspensions at designated storage periods*

| Strain | Time of storage (months) | Viability | Time of storage (months) | Viability |
|---|---|---|---|---|
| ATCC-9340 | 3 | +[a] | 41 | + |
| TD-60 | 3 | + | 45 | + |
| MK-05 | 3 | + | 40 | + |
| LW-41 | 2 | + | 45 | + |
| MR-40 | 2 | + | 45 | + |
| JG-04 | 2 | + | 39 | + |
| DN-14 | 2 | + | 39 | + |
| DV-85 | 2 | + | 39 | + |
| MV-21 | NT[b] | | 43 | + |
| BR-16 | 1 | + | 38 | + |
| PT-07 | NT | | 37 | + |
| AG-17 | (1 day) | + | 37 | + |
| MR-72 | NT | | 36 | + |
| AE-57 | NT | | 36 | + |
| DF-89 | NT | | 36 | + |
| PP-00 | NT | | 36 | + |
| TP-81 | NT | | 41 | + |
| LS-18 | NT | | 36 | + |

[a] Luxuriant growth of undiluted 0.1-ml suspension on freshly prepared B-G medium.
[b] Not tested.

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