

FIG. 2. Electron micrograph of chromium-shadowed base of a flagellum. There can be seen simply a structureless, spherical granule. $\times 60,000$.

LIQUID NITROGEN FREEZING IN MICROBIOLOGICAL ASSAY SYSTEMS

II. PRESERVATION OF *SARCINA LUTEA* FOR ANTIBIOTIC ASSAYS

E. M. STAPERT, W. T. SOKOLSKI, W. M. KANESHIRO, AND R. J. COLE

Control Laboratories, The Upjohn Company, Kalamazoo, Michigan

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The storage of *Lactobacillus leichmannii* in liquid nitrogen for direct use in the vitamin B₁₂ assay was described by Sokolski, Stapert, and Ferrer (Appl. Microbiol. **12**:327, 1964). This note describes a different approach on treatment of data for stability of a test organism. A stable suspension of *Sarcina lutea* ATCC 9341, a bacterium used in many antibiotic assays, was obtained with storage in liquid nitrogen. Criteria for stability were slope variations in day-to-day responses to lincomycin and the appearance of the inhibition zones on the test plates.

A suspension of *S. lutea* was prepared in BBL Antibiotic Assay Broth as described by Grove and Randall (*Assay methods of antibiotics*, Medical Encyclopedia, Inc., New York, 1955, p. 14-15). A portion of the suspension was distributed to 1.2-ml ampoules (Breeder Kimax, Owens-Illinois Co., Toledo, Ohio). The ampoules were sealed and immersed directly into liquid nitrogen. When ready for use, the ampoules were rapidly thawed

by immersion and agitation in a water bath (40 C). The remaining suspension was stored at 4 C and was used as inoculum on each assay day. The nonfrozen (4 C) suspension was used with both a constant inoculum rate of 0.7% and with a varying inoculum rate which was increased progressively after 1 week of storage to obtain approximately the same zone diameters per dose throughout the study. The inoculum rate for the frozen suspension was kept constant at 0.7% in all assays. All assay plates were made with 21 ml of Penassay Base Agar (Difco) layers and 4 ml of Penassay Seed Agar (Difco) seeded with 0.7% *S. lutea* suspension as the constant inoculum. The varying inoculum rates with the unfrozen suspension were 0.7% up to 5 days of storage, 0.8% at 5 and 7 days, 1.0% at 34 days, 1.2% at 42 days, and 1.4% at 54 and 61 days. Six-point standard curves were run on each assay, with lincomycin concentrations at 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 $\mu\text{g/ml}$.

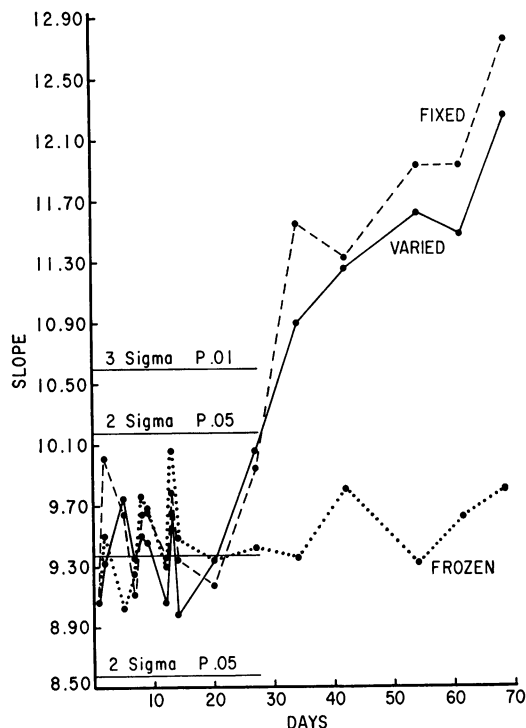


FIG. 1. Slopes calculated for six-point lincomycin standard curve.

The suspension of *S. lutea* stored in liquid nitrogen was stable throughout the 68-day experiment (Fig. 1). The dose-response slope for the suspensions stored at 4 C changed markedly after 27

days. The inhibition zone edges were fuzzy and became progressively more difficult to read with these inocula after about 8 days, although the zone edges were sharp throughout the experiment with the frozen inoculum. A technician without considerable experience in measuring zone diameters would have difficulty estimating the potencies with the unfrozen inocula after 14 days of storage. An inexperienced technician might have difficulty reading the fuzzy zone edges with good reproducibility among zones.

Since no significant differences were evident for the first 27 days or 12 assay days among the fixed, varied, and frozen inocula slopes, these data were pooled. The mean, 2 sigma limits ($P = 0.05$), and the upper 3 sigma limit ($P = 0.01$) on the graph are from the pooled data. Five additional assay days indicated that the slopes with the unfrozen inocula were significantly different from the frozen inoculum. The unfrozen inocula slopes fell outside the 3 sigma limit, while the frozen inoculum remained within 2 sigma limits. These data show that the inoculum frozen in liquid nitrogen provided a more uniform slope over the 68-day period. Data on *Staphylococcus aureus* ATCC 6538P indicate that this suspension is stable for the neomycin assay after storage for 15 months.

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PLAQUE FORMATION BY PSITTACOSIS VIRUS

EDMUND H. KOZIKOWSKI AND NICHOLAS HAHON

U.S. Army Biological Laboratories, Fort Detrick, Frederick, Maryland

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Although the production of plaques on cell monolayers has been reported for a considerable number of viral agents (Cooper, *Advan. Virus Res.* **8**:319, 1961), it has been demonstrated for only one member of the psittacosis group, meningopneumonitis virus (Tamura and Higashi, *Ann. Rept. Inst. Virus Res. Kyoto Univ.* **2**:57, 1959; Higashi and Tamura, *Virology* **12**:578, 1960). This preliminary communication describes plaque formation by another member, psittacosis (Borg) virus.

Cultures of an established cell line, McCoy, originally derived from human synovial tissue

(Fernandes, *Z. Zellforsch. Mikroskop. Anat.* **50**:433, 1959) and shown to be 99% susceptible to infection with psittacosis virus (Hahon and Nakamura, *in press*), were suspended in nutrient medium composed of mixture 199 with 0.5% lactalbumin hydrolysate (LAH), 10% heat-inactivated calf serum, 50 μ g of streptomycin, and 75 μ g of kanamycin. A suspension (5 ml) containing 1.5×10^6 cells was dispensed into 2-oz bottles (Sani-Glas, Brockway Glass Co., Brockway, Pa.), capped, and incubated at 35 C. Continuous cell monolayers were formed, usually within 48 hr. These were washed once with 10