Stabilities of Lyophilized *Staphylococcus aureus* Typing Bacteriophages

CHARLES H. ZIERDT

Microbiology Service, Department of Clinical Pathology, Building 10, Room 2C385, National Institutes of Health, Bethesda, Maryland 20892

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*Staphylococcus aureus* bacteriophages (25 phages) were lyophilized in aliquots 12 to 18 years ago and stored in vacuo at −20°C. Eight viruses each lost one log titer, while seventeen retained the original titers. The use of lyophilized phages provided more reproducible phage typing and reduced by 75% the complexity and cost. This important test is thus made feasible for more laboratories.

Bacteriophage typing of *Staphylococcus aureus* has provided the only practical means of reliably fingerprinting strains of this leading nosocomial pathogen for hospital surveillance and epidemiological research and control (1-3, 7). But only a few reference laboratories can afford to assign one or more persons to this viral technology. However, the time and expertise requirements can be reduced by 75% or more if lyophilized and titered virus stocks are made available. The use of a phage applicator (5) further reduces the time needed.

There is a paucity of information on the long-term viability of preserved bacteriophages. For this study, the viabilities of *S. aureus* phages stored in vacuo at −20°C for 12 to 18 years were tested by previously described methods (3). The lyophilized pellet was suspended in 3 ml of tryptic soy broth. This was considered the stock suspension, and the titer was established as the highest decimal dilution of the stock suspension producing confluent lysis of the specific phage host strain of *S. aureus* on typing agar. While a plaque count is a more sensitive method for estimating viral numbers, confluent lysis is the standard method of determining the titers of typing phages. Of the 25 phages tested, 8 (32%) lost one dilution (10-fold) of titer, while 17 (68%) retained the original postlyophilization titers (Table 1). An annual titer determination should be adequate to indicate a slow loss of titer when it occurs.

Continuous viral propagation and titration were eliminated after the viral stocks were prepared by previously described methods (3, 6). The phages and host strains can be obtained from most reference centers. Other advantages of lyophilized stocks are avoidance of subtle or major virus mutations which occur during continuous phage propagation and assurance of exactly the same dose and quality of phage lysate for each *S. aureus* phage typing run. By having in-house phage typing capability, more strains can be typed and typing quality and speed can be improved.

### LITERATURE CITED


### TABLE 1. Stabilities of lyophilized *S. aureus* international typing bacteriophages

<table>
<thead>
<tr>
<th>International phage designation</th>
<th>Postlyophilization* phage titer</th>
<th>Titer of suspended phage</th>
<th>No. of yrs in lyophilized state at −20°C</th>
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* Reciprocal of titer.