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Stabilities of Lyophilized *Staphylococcus aureus* Typing Bacteriophages

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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.

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

International phage designation	Postlyophilization[a] phage titer	Titer of suspended phage	No. of yrs in lyophilized state at −20°C
3A	10,000	1,000	18
3B	100,000	100,000	18
3C	100,000	100,000	17
6	100,000	100,000	18
7	10,000	10,000	18
29	10,000	10,000	18
42B	10,000	10,000	13
42D	100,000	10,000	18
42E	10,000	10,000	18
44A	100,000	10,000	18
47	100,000	100,000	18
52	10,000	10,000	18
52A	100,000	10,000	17
53	10,000	10,000	18
54	100,000	100,000	18
55	10,000	10,000	15
71	10,000	10,000	13
75	100,000	100,000	18
77	10,000	10,000	12
79	10,000	10,000	18
80	10,000	1,000	18
81	10,000	1,000	18
83	100,000	10,000	12
86	10,000	10,000	12
187	100,000	10,000	17

[a] Reciprocal of titer.

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.

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