

Sensitivity of Phage Replication Assay to Culture Conditions

The two-point method to estimate the growth rate of phage for the Phage Replication Assay is an approximation intended to facilitate the in vitro screening of potentially many different phages for their therapeutic potential. The estimation method assumes that phage numbers are increasing exponentially at a constant per capita rate, ρ . There are two possible concerns with this assumption. First, cultures of infections are often highly synchronous at the outset. This synchrony occurs because the entire population of phage used to inoculate a culture is typically added at one time. The life history of most phages involves a highly uniform time lag between adsorption and burst of the infected cell (typically on the order of 20-40min). Therefore, a culture will typically exhibit oscillations in the density of extracellular phage for some initial phase, as the synchronized culture goes through periods when most infections have just occurred, then most infections are in the lag phase, then most burst, and so on. Attempts to estimate phage growth rate during this early period of synchrony will give misleading results. It is thus important to conduct the assay over a long enough interval that the population of infections has become largely asynchronous. A second concern is that, if phage are replicating in a growing population of bacteria, the rate of replication of the phage will change as the density of the bacterial population changes. As we illustrate here, this change in bacterial density has a modest effect on the relative *PRA* comparisons but can have a marked effect on absolute *PRA* values.

The rate at which a population of lytic phage replicates in a growing population of bacteria depends on phage density, P , on the density of the bacterial population, N , and on at least three parameters: the rate at which the phage adsorb to sensitive bacteria, δ (ml x cells x phage per hour), the latent period, λ (hours) and the burst size, β (particles). If we assume the bacteria are growing at constant rate r , the rate of change in the densities of the bacterial and phage populations in liquid culture are given by.

$$dN/dt = rN - \delta NP$$

$$dM/dt = \delta NP - M(t-\lambda)$$

$$dP/dt = M(t-\lambda)\beta - \delta NP,$$

where M is the density of phage infected bacteria that have yet to burst and $M(t-\lambda)$ is that density λ time units earlier. We are assuming that burst is an instantaneous process, that there is no mortality of phage or bacteria other than included in r and that due to phage, and there is no lysis inhibition, where infections with multiple phage increase the length of the latent period. We also assume that the ill-fated population of infected bacteria (M) does not replicate. These coupled differential equations were iterated numerically as discrete equations, incrementing them every 0.004hr.

To compare phage growth in this model to the pure exponential growth model assumed for the calculation of ρ , we consider a community with an initial density of 10^3 phage and 10^6 bacteria, a burst size of 100 particles, latent periods of 20, 30, and 40 minutes and a phage adsorption rate of 10^{-8} . These parameter values are in the range estimated for lytic phage [1, 2].

The rate of phage doubling [$\log_2(\rho)$] is sensitive to the time at which the calculation is made (Table A.1); the estimated value increases with time because the increasing bacterial density (which exceeds the phage density throughout the 3 hrs) reduces the phage generation time by increasing the rate at which phage encounter hosts. Provided that estimates of ρ or $\log_2(\rho)$ are compared from the same time points, the ranking of phages is largely unaffected, but differences between phages increase as cell density increases. This simulation thus reveals that the comparison of *PRA* values across different phages needs to be made under similar conditions of cell physiology and cell density, but that information about *PRA* values at different cell densities (and other conditions) is also desirable before reaching any conclusions about the general superiority of one phage over another. To obtain reliable estimates, it may be important to grow cells to the desired density before adding the phages, to avoid the complication that faster-growing phages will reduce bacterial densities more than slow-growing phages before the time at which ρ is estimated.

References

1. L Chao, BR Levin, FM Stewart: **A complex community in a simple habitat: an experimental study with bacteria and phage.** *Ecology* 1977, **58**:369-378.
2. BR Levin, FM Stewart, L Chao: **Resource - limited growth, competition , and predation: a model and experimental studies with bacteria and bacteriophage.** *American Naturalist* 1977, **97**:3-24.

Table A.1. Simulation Results: The phage replication rate $\log_2(\rho)$

Parameters: r = bacterial growth rate/hr; λ = latent period in minutes, $\delta = 10^{-8}$, $\beta = 100$. These estimates of ρ used the end point method described in Methods, where $\rho = [P(t+\varepsilon)/P(t)]/\varepsilon$, and $P(t+\varepsilon)$ and $P(t)$ are the densities of phage at time t and at $t+\varepsilon$ hours. In these calculations, the interval ε was taken as the equivalent of 0.004hr, and t was either 1hr or 3hr.

Table A.1

r	λ	$\log_2(\rho)$		bacterial density	
		1hr	3hr	1hr	3hr
1	40	1.4	3.4	2.7×10^6	2.0×10^7
1	30	1.4	4.3	“	“
1	20	1.9	5.7	“	“
2	20	3.0	13.0	7.4×10^6	3.5×10^8