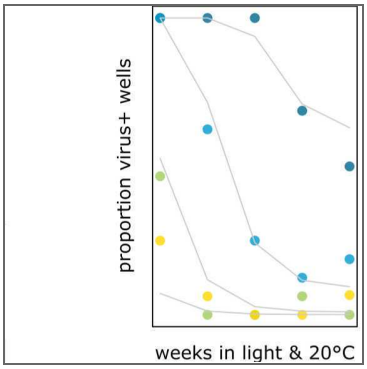

Modified survival (mS) assay V.3

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MANUSCRIPT CITATION:
Lievens, Agarkova, Dunigan, Van Etten, and Becks (2023) Efficient assays to quantify the life history traits of algal viruses. *Applied & Environmental Microbiology.* doi.org/10.1128/aem.01659-23

ABSTRACT

This protocol describes a modified survival assay, as presented in the paper "*Efficient assays to quantify the life history traits of algal viruses*" (Lievens et al. 2023, *Applied & Environmental Microbiology*, doi.org/10.1128/aem.01659-23). It is designed to quantify the specific infectivity and mortality rate of chloroviruses (large, lytic dsDNA viruses). The protocol should also be applicable to other lytic viruses that are large enough to be counted by flow cytometry. For viruses that are not large enough to be counted by flow cytometry, this protocol could be adjusted to start with different MOIs. It would then measure the mortality rate only.

Compared to a classic survival assay (or decay assay, inactivation assay, ...), this protocol uses predetermined virion concentrations and miniaturization to increase efficiency. Briefly, suspensions containing 50000, 5000, 500, or 50 virions/ml are prepared for each virus, these suspensions are split into aliquots, and independent aliquots are used to do an MPN-like assay after 0, 7, 14, ... days. The MPN-like assay involves distributing a 50000, a 5000, a 500, and a 50 virions/ml aliquot across 16 liquid cultures of host cells (algae) and observing the number of cultures that are lysed. Non-linear statistical models are then used to estimate the initial concentration and decline of infectious virions. See the associated manuscript for a full description and schematic figures, as well as the statistical model.

Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID: 74272

MATERIALS

For this protocol, we use:

- to store virus samples: 15ml PP centrifuge tubes from Sarstedt (ref. 62.554.502, Sarstedt, DE) - *don't interfere with viruses**
- culture plates: 96-well polystyrene tissue culture plates from Techno Plastic Products (ref. TPP92696, Techno Plastic Products, CH) - *don't interfere with viruses*, can withstand centrifugation, and let light through in the survival conditions*
- deep well plates: 96-well 2.2ml PP deep well plates from VWR (ref. 732-0585, VWR International, USA) or Abgene (ref. AB0932, Thermo Scientific, DE) - *don't interfere with viruses**
- transparent seals: PCR plate seals from VWR (ref. 732-3212, VWR International, USA) - *very adhesive and let light through in the survival conditions*

**We find that in many storage containers, the virion concentration decreases over time (EJPL, unpublished data). This includes many types of PCR plates, eppis, and centrifuge tubes. The effect may be because virions adsorb to certain plastics. Any new storage container should be tested before use!*

Also necessary:

- up to 47 virus samples
- an algal culture in a growth phase that can be infected by the viruses
- centrifuge with a rotor for plates
- vortex
- multichannel pipettes
- haemocytometer and microscope, or some other way of counting algae
- Parafilm

BEFORE START INSTRUCTIONS

This version is for two virus plates, which corresponds to max. 47 different viruses, and 5 time points (including time point 0). It is assumed that the virion concentration of the virus samples is known (see dx.doi.org/10.17504/protocols.io.6qpvr6q93vmk/v1).

Use sterile techniques throughout this protocol. Using materials that don't bind viruses is important; see *Materials* for more information.

Definitions:

- BBM = modified Bold's Basal Medium, see the associated publication
- survival conditions = the conditions under which survival is being tested (e.g. a specific temperature)
- growth conditions = the conditions that allow algae to grow (e.g. 20°C, constant light, on an orbital shaker)
- culture plates = 96-well flat-bottomed tissue culture plates (see *Materials*)
- deep well plates = 96-well 2.2ml PP deep well plates (see *Materials*)
- transparent seal = transparent PCR plate seal (see *Materials*)

time point 0 - prepare & aliquot suspensions

1 Mix virus suspensions at 50000, 5000, 500, or 50 virions/ml.

1.1 In two deep well plates ("1" and "2"), mix viral suspensions so they have a volume of 1.5ml and 50000, 5000, 500, or 50 virions/ml. Avoid reusing tips for virus pipetting; reusing tips leads to lower accuracy (possibly because viruses bind to the plastic) (EJPL, unpublished data).
Notes: If the virus samples are highly concentrated, it may be necessary to make preparatory dilutions.

	A	B	C	D	E	F	G	H
	plate	column	row	virus	concentration (virions/ml)	preparatory dilution (1:...)	virus to add (µl)	BBM to add (µl)
	1	1	A	NE-JV4	50000	1	473	1027
	1	1	B	NE-JV4	5000	1	47.3	1453
	1	1	C	NE-JV4	500	1	4.73	1495
	1	1	D	NE-JV4	50	10	4.73	1495
	1	1	F	MA-1E	50000	1	18.6	1481

	A	B	C	D	E	F	G	H
	1	1	G	MA-1E	5000	1	1.85	1498
	1	1	H	MA-1E	500	10	1.85	1498
	1	2	A	MA-1E	50	100	1.85	1498
	1	2	C	PBCV-1	50000	1	1.05	1499
	1	2	D	PBCV-1	5000	10	1.05	1499
	1	2	E	PBCV-1	500	100	1.05	1499
	1	2	F	PBCV-1	50	1000	1.05	1499
	...	...	...	...	...		...	...
	1	12	G	neg. ctrl.				1500
	1	12	H	neg. ctrl.				1500

Example pipetting scheme for one deep well plate.

1.2 Mix the deep well plates by using a 1000µl multichannel pipette to pipette each column up and down 3x.

2 Prepare the **virus plates**.

2.1 Using a multichannel pipette, aliquot 200µl from deep well plate "1" into five culture plates. These are the virus plates "1". Do the same for deep well plate "2" and the virus plates "2".

2.2 Seal all but one of the virus plates "1" and all but one of the virus plates "2" with a transparent seal, and store under the survival conditions.

time point 0 - quantify lysis abilities with an MPN-like assay

3 Prepare an intermediate algal suspension.

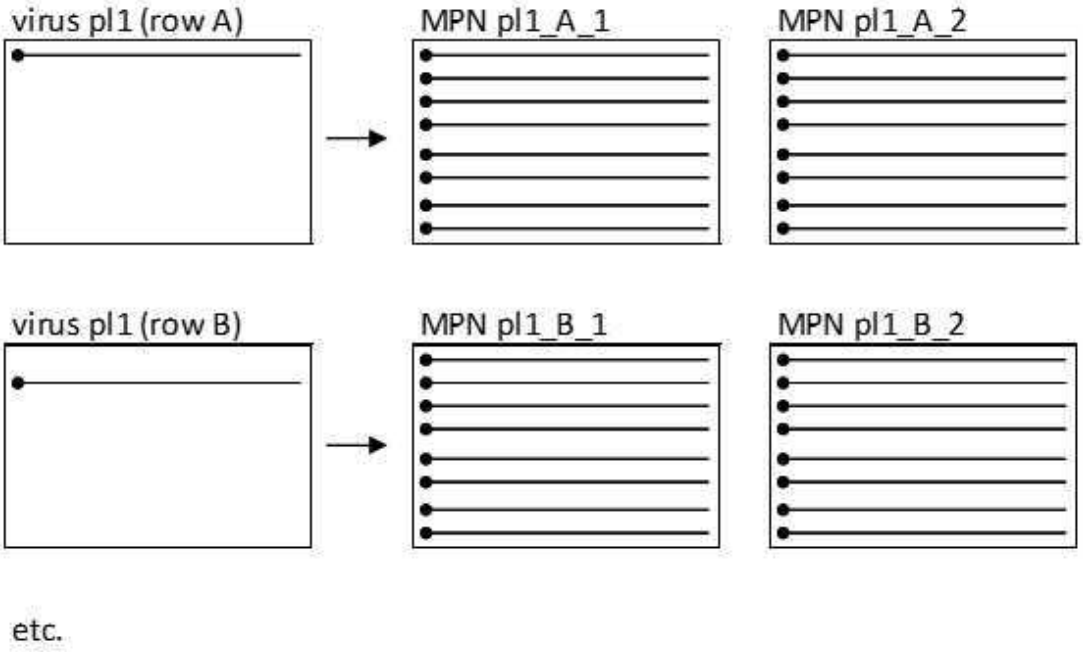
3.1 Count the concentration of algal cells in the algal culture using a haemocytometer.
Notes: If necessary, centrifuge the algae for 5min at 4500rpm, gently pour off the supernatant, resuspend the pellet, and count the concentration of algal cells in the pellet.

3.2 Using the algal culture and BBM, prepare 590ml of an intermediate algal suspension at $1.05*10^6$ cells/ml.
Background info: This concentration is late exponential phase for our algae; the idea is that the algae are concentrated enough for the viruses to lyse quickly, but aren't yet in stationary phase.

4 Prepare the **MPN plates**.

4.1 Aliquot 190µl algae into each well of 32 culture plates. These are the MPN plates.

4.2 Using a multichannel pipette (ideally a 12-channel multichannel), distribute 10µl from the each row of the remaining virus plate "1" into two MPN plates as shown. Do the same for the remaining virus plate "2". It is possible to use the same tips for all the aliquots of a given row. Label each MPN plate with the virus plate number ("pl1" or "pl2"), virus plate row, and suffix 1 or 2.



Background info: This step makes it clear why the initial concentrations maximize the power of the MPN-like assay:

- 50000 virions/ml concentration = 500 virions in 10µl -> if the initial proportion of infectious virions (the specific infectivity) is 0.001, half of the 16 replicate wells will be virus-positive at time point 0
- 5000 virions/ml concentration = 50 virions in 10µl -> if the initial proportion of infectious virions (the specific infectivity) is 0.01, half of the 16 replicate wells will be virus-positive at time point 0
- 500 virions/ml concentration = 5 virions in 10µl -> if the initial proportion of infectious virions (the specific infectivity) is 0.1, half of the 16 replicate wells will be virus-positive at time point 0
- 50 virions/ml concentration = 0.5 virions in 10µl -> if the initial proportion of infectious virions (the specific infectivity) is 1, half of the 16 replicate wells will be virus-positive at time point 0

4.3 Close the MPN plates with their lids, wrap the edges in Parafilm to reduce evaporation, and store them under the growth conditions.

5 After 4 days, check the algal growth in the MPN plates. This can be done by eye, by measuring optical density at 680nm, etc.

6 Collect one virus plate "1" and one virus plate "2" from the survival conditions. Vortex the plates and spin down.

7 Use these virus plates to repeat steps 3-5.