

FVIC (Frequency of Visibly Infected Cells) Protocol

Jennifer Brum

Abstract

Purpose: This protocol describes how to pellet bacterial cells onto grids and stain them so that viruses can be visualized within the cells using transmission electron microscopy. Data obtained from these grids can be used to calculate the frequency of visibly infected cells (FVIC), the frequency of infected cells (FIC), the fraction of mortality due to viral lysis (FMVL), and burst size.

Citation: Jennifer Brum FVIC (Frequency of Visibly Infected Cells) Protocol. **protocols.io**

dx.doi.org/10.17504/protocols.io.dbp2mm

Published: 21 Jan 2016

Guidelines

Materials required:

- glow discharge apparatus
- ultracentrifuge and rotor capable of 55000 xg
- pollyallomer ultracentrifuge tubes with epoxy in the bottom to provide a flat surface
- EM grid-grade forceps
- EM grids (formvar coated 200 mesh copper grids)
- paper dividers from between commercially packaged filters
- double-sided tape
- razor blade
- uranyl acetate
- ultra-purified water such as Milli-Q water
- petri dish
- 4x 2 ml microcentrifuge tubes with screw caps
- 4 ml plastic culture tube with cap
- small stir bar
- stir plate
- filter paper (cut into wedges)
- 0.02 μm syringe filter and syringe (3-5 ml)
- lab coat, respiratory protection (mask), eye protection
- timer
- transmission electron microscope

Applicable References:

Proctor and Fuhrman (1990) – The original description of the FVIC method.

Binder (1999) – The equations used to calculate FIC and FMVL, and the assumptions inherent in those equations.

Brum et al. (2005) – An example of a use of the FVIC method to investigate viral induced mortality of

bacteria in an aquatic ecosystem.

Binder, B. (1999) Reconsidering the relationship between virally induced bacterial mortality and the frequency of infected cells. *Aquatic Microbial Ecology* 18: 207-215.

Brum, J.R., Steward, G.F., Jiang, S.C., and Jellison, R. (2005) Spatial and temporal variability of prokaryotes, viruses, and viral infections of prokaryotes in an alkaline, hypersaline lake. *Aquatic Microbial Ecology* 41: 247-260.

Proctor, L.M., and Fuhrman, J.A. (1990) Viral mortality of marine bacteria and cyanobacteria. *Nature* 343: 60-62.

Protocol

Preparing the grids

Step 1.

On the same day of grid prep, use glow discharge to render grids hydrophilic.

Preparing the grids

Step 2.

Cut a circle out of a paper filter divider such that it has a diameter slightly smaller than the interior diameter of the ultracentrifuge tubes.

Preparing the grids

Step 3.

Cut small rectangles (3 mm x 1 mm) of double-sided tape using a razor blade.

Preparing the grids

Step 4.

Attach the discharged grids (shiny-side-up) to the circle of paper using the double-sided tape such that only the edge of the grids contacts the tape (Figure 1)

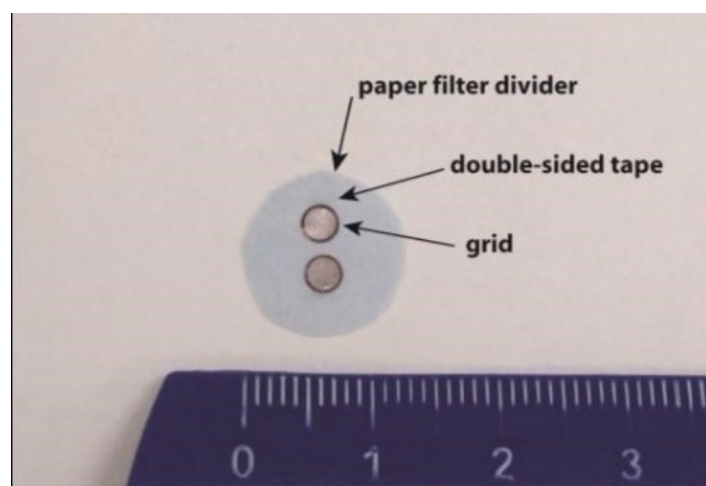


Figure 1: Grids attached to the paper filter divider with double-sided tape.

Preparing the grids

Step 5.

Insert the paper with the attached grids into the ultracentrifuge tube grids-side-up (Figure 2)

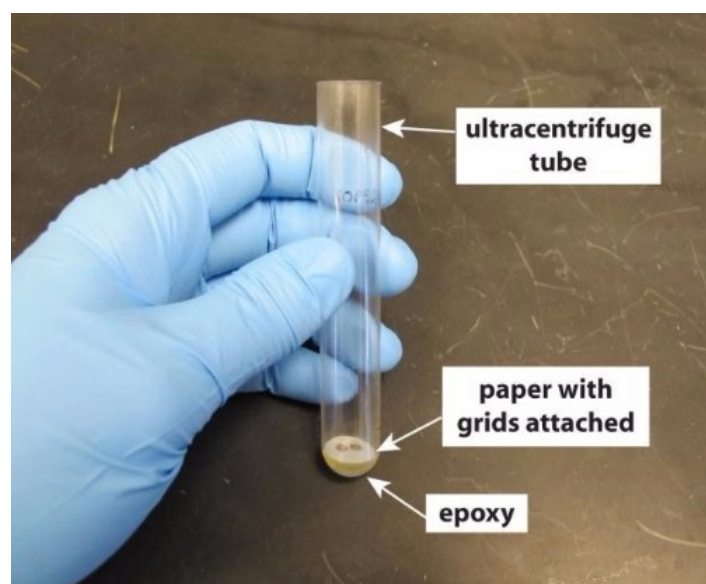


Figure 2. Grids inserted into the bottom of the ultracentrifuge tube.

Preparing the grids

Step 6.

Add your sample to the ultracentrifuge tube ensuring that there are no air bubbles under the circle of paper.

📌 NOTES

VERVE Team 12 Aug 2015

Note: ca. 20 ml of sample is required for samples with bacterial concentrations of 10^7 ml⁻¹. Adjust the sample volume according to the bacterial concentration in your samples.

Preparing the grids

Step 7.

Centrifuge samples using the following conditions (you can use a different rotor as long as it can reach the appropriate g-force):

Rotor: Beckman SW 40 Ti

Temperature: 4°C

Time: 1 hr

Speed: 20000 rpm (average force = 55000 xg)

📌 NOTES

VERVE Team 12 Aug 2015

Balance the rotor exactly with another sample or dummy sample.

Preparing the grids

Step 8.

After centrifugation, you can remove the sample and add more sample for additional centrifugation runs to achieve the required volume .

Preparing the grids

Step 9.

Make 2 ml of 0.5% uranyl acetate with Q-water in a 4 ml culture tube using a stir bar to mix the sample on a stir plate for 30 minutes to 1 hour.

DURATION

01:00:00

NOTES

VERVE Team 12 Aug 2015

Do not turn on the heat!

VERVE Team 12 Aug 2015

0.01 g uranyl acetate + 2 ml Q-water = 0.5% uranyl acetate

VERVE Team 12 Aug 2015

Caution: uranyl acetate is toxic so read the MSDS and use a lab coat, eye protection, respiratory mask, and gloves when handling it in powdered form

Preparing the grids

Step 10.

Filter the uranyl acetate solution through a 0.02 μm syringe filter into a 2 ml screw-cap tube.

NOTES

VERVE Team 12 Aug 2015

This removes any particles of uranyl acetate that have not fully dissolved.

Preparing the grids

Step 11.

Fill 3x 2 ml screw-cap tubes with Q-water.

Preparing the grids

Step 12.

Using tweezers, remove the piece of paper with the attached grids from the ultracentrifuge tube and place in a petri dish.

Preparing the grids

Step 13.

Using tweezers, remove a grid from the piece of paper (make sure the tape is not still attached to the grid).

Preparing the grids

Step 14.

Immerse the grid into the 0.5% uranyl acetate solution for 30 seconds (Figure 3)

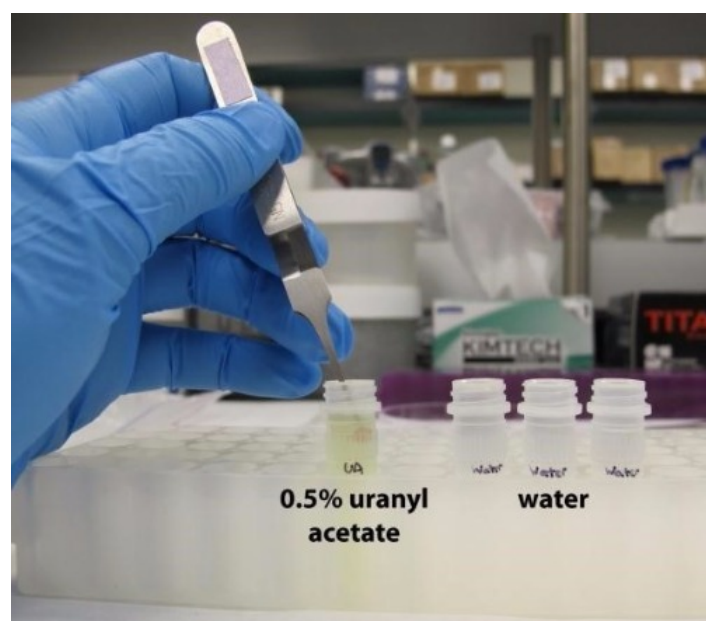


Figure 3. Immersing the grid in the uranyl acetate solution.

🕒 DURATION

00:00:30

Preparing the grids

Step 15.

Immerse the grid into the 1st tube of Q-water for 10 seconds.

🕒 DURATION

00:00:10

Preparing the grids

Step 16.

Immerse the grid into the 2nd tube of Q-water for 10 seconds

🕒 DURATION

00:00:10

Preparing the grids

Step 17.

Immerse the grid into the 3rd tube of Q-water for 10 seconds.

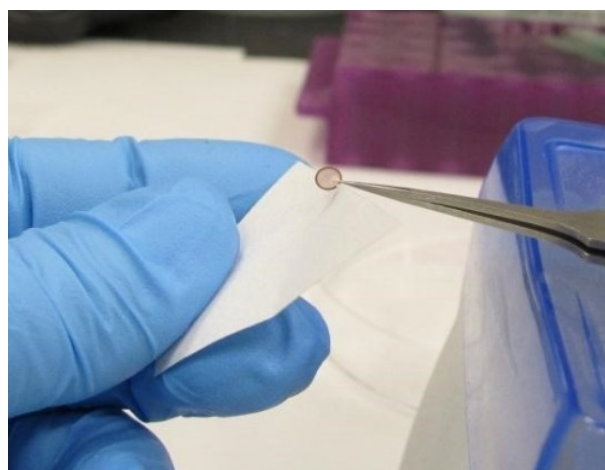
🕒 DURATION

00:00:10

Preparing the grids

Step 18.

Wick away liquid from the grid using a wedge of filter paper placed at the edge of the grid (Figure 4)



Preparing the grids

Step 19.

Place the grid in a grid box and allow to dry overnight, then store the grid in a desiccator until analysis.

📌 NOTES

VERVE Team 12 Aug 2015

Do not put wet grids in a desiccator immediately as this can create “cracking” of the stain.

Grid analysis

Step 20.

Using a transmission electron microscope with 100 kV accelerating voltage, examine a grid at 40000X.

Grid analysis

Step 21.

Look at 800 cells per grid. Count how many cells have >5 viruses assembled within the cell (Figure 4)

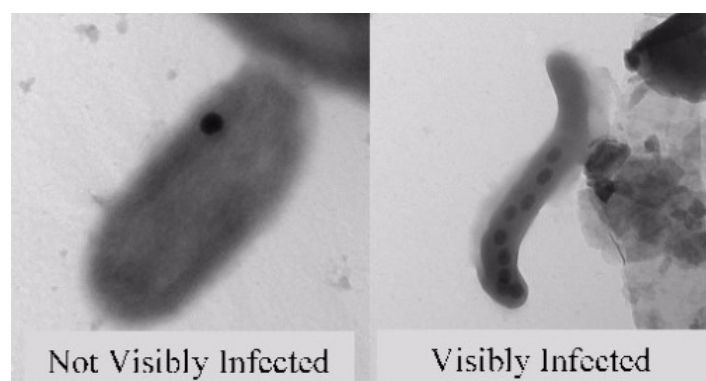


Figure 4. Examples of cells that would be counted as visibly infected and not visibly infected.

📌 NOTES

VERVE Team 12 Aug 2015

Greater than 5" rule prevents you from mistaking cellular structures or extracellular viruses from intracellular viruses.

Grid analysis

Step 22.

Take a digital micrograph of all visibly infected cells.

Grid analysis

Step 23.

Calculate the frequency of visibly infected cells (FVIC):

$$FVIC = \frac{(\text{number of visibly infected cells})}{(\text{total number of cells examined})}$$

Grid analysis

Step 24.

Calculate the frequency of infected cells (FIC):

$$FIC = (7.1 \times FVIC) - (22.5 \times FVIC^2)$$

Grid analysis

Step 25.

Calculate the fraction of mortality from viral lysis (FMVL):

$$\text{FMVL} = \frac{[\text{FIC} + (0.6 \times \text{FIC}^2)]}{[1 - (1.2 \times \text{FIC})]}$$

Grid analysis

Step 26.

Determine burst size: Using the micrographs of visibly infected cells, count how many viruses are present inside of cells that are full of viruses.

🔗 NOTES

VERVE Team 12 Aug 2015

*Caveats:

- some cells can burst before they are full of viruses (thus, this is an overestimate of burst size)
- it may be impossible to count the total number of viruses within larger cells because they are overlapping one another (thus, this is an underestimate of burst size)

Grid analysis

Step 27.

Other information that can be obtained using ImageJ or other image analysis software:

- dimensions of infected cells
- dimensions of intracellular viruses

Warnings

*Caution: uranyl acetate is toxic so read the MSDS and use a lab coat, eye protection, respiratory mask, and gloves when handling it in powdered form.