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PCTE Protocol for VLP enumeration

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Ken Stedman[1], Geoffrey Diemer[1], Jennifer E Kyle[1]

[1]Stedman Lab

VERVE Net

Ken Stedman
Portland State University

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Abstract

An alternative epifluorescence microscopy (EFM) technique for virus enumeration has been developed using polycarbonate Track Etch (PCTE) filter membranes in place of aluminum oxide Anodisc membranes. The established EFM technique, first developed by Hennes and Suttle in 1995 and later expanded upon by Noble and Fuhrman in 1998, uses supported 20 nm pore-size Anodisc filter membranes to determine virus abundance in natural environments. Increased price and sporadic availability of Anodisc filters stimulated the evaluation of alternative filters for use in the procedure. The feasibility of using 30 nm pore-size PCTE filters for virus enumeration was assessed using the Anodisc filter procedure as a control. Although virion particle counts are slightly less precise using PCTE filters, they offer a substitute for Anodiscs while requiring only minor adjustments to the established protocol. Per slide, the PCTE costs approximately ten times less to prepare than the Anodisc-based method.

1 A 200 nm pore, 25mm diameter PVDF support filter is first placed onto a fritted glass base to provide an even surface for the diaphanous PCTE filter.

2 The PVDF filter is saturated with 200 µL of virus-free water after being placed on the fritted glass base.

Note

Slight vacuum suction may be applied to absorb water drops into the PVDF support filter.

3 The PCTE filter is then carefully mounted on top of the damp support filter.

Note

Placing the PCTE filter on the support filter with a slight overlap is preferable, if possible, so that it is easier to remove the PCTE filter after the process is finished.

4 The funnel is then mounted on the fritted base and clamped in place to secure the support and PCTE filters.

5 The PCTE filter is then stained with Sudan black B while held in place between the fritted base and funnel.

Protocol

NAME

PCTE filter staining

CREATED BY

Ken Stedman

PREVIEW

5.1 1 mL of 4 µg/mL Sudan black B solution is added to the funnel and is drawn through the filter at a vacuum pressure of -70 kPa.

5.2 Suction is applied until the PCTE filter appears dry.

Note

The flow rate through 30 nm pore PCTE filters is approximately 0.1 mL/min, at -70 kPa using a 25mm diameter funnel.

6 The virus solution is then added to the funnel and drawn through the stained PCTE filter at -70 kPa.

Note

It is preferable to filter a volume of at least 0.5 mL of virus solution as smaller volumes may not uniformly cover the membrane surface during filtration.

7 When the virus particles have been extracted from the suspension, vacuum is released and 0.5 mL of a 1:400 SYBR Gold solution is added to the funnel.

Note

The SYBR Gold solution is prepared using SM buffer (100mM NaCl, 10mM $MgCl_2$ and 50mM Tris-Base, adjusted to pH 8.0) or TE Buffer (10mM Tris-Base, 1mM EDTA, adjusted to pH 8.0) to maintain a pH between 7.5 and 8.3, as pH outside this range adversely affects fluorescence of the SYBR Gold stain (Invitrogen).

8 The PCTE filter, now with viruses on the surface, is allowed to stain for 10 minutes in the dark, without vacuum.

00:10:00

9 After 10 minutes, vacuum is re-applied to draw the SYBR Gold solution through the filter.

10 One mL of SM or TE buffer is then drawn through the filter to rinse away excess stain and to remove viruses that may have stuck to the walls of the funnel during staining.

11 Vacuum is maintained until the wash solution has completely passed through the PCTE filter.

12 While the filter wash process is underway, a 5 µL drop of 1X antifade (Patel et al. 2007) is placed on the surface of a clean microscope slide, and an additional 5 µL drop is placed on a cover slip.

13 When the wash is complete, vacuum is released and the PCTE filter is carefully removed from the support filter with forceps and waved very gently in the air a few times to dry.

14 The PCTE filter is then placed face-up on the 5 µL drop of antifade on the surface of the slide.

15 The cover slip, with the 5 µL drop of antifade, is placed drop-side down on top of the PCTE filter.

Note

Using more than 5 µL of antifade will significantly increase wrinkling of the PCTE filter on the glass slide.

16 The cover slip is gently pressed down to remove air bubbles, to smooth small wrinkles in the filter, and to saturate the filter with antifade solution.

Note

As 30 nm pore PCTE filters are exceedingly thin, small wrinkles will shift the focal plane when the slide is being viewed under the microscope, making counting difficult. It is therefore recommended that the PCTE filter is pressed as flat as possible underneath the cover slip before viewing.

17 The PCTE slide should be viewed by EFM within a few hours after preparation as fading occurs even when stored at -20ºC.