OptiPrep™ Mini-Review MV04

Purification and analysis of retroviruses

- OptiPrep™ is a sterile 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- This Mini-Review briefly reviews the gradient technology for the purification and analysis of retroviruses (Sections 1-3). Principally however it provides a bibliography (Section 4) of all those papers reporting the use of OptiPrep™ in the purification and analysis of retroviruses. These viruses are:

    - Human immunodeficiency virus-1 (HIV-1): Application Sheet V34
    - Lentivirus vectors: Application Sheet V34
    - Moloney murine leukaemia virus: Application Sheet V33
    - Human T-cell lymphotropic virus (HTLV-1): Application Sheet V31
    - Human endogenous retrovirus (HERV-H): Application Sheet V31
    - Foamy viruses (*Spumaviridae* genus): Application Sheet V35
    - Mason-Pfizer Monkey virus: Application Sheet V30
    - Rous sarcoma virus: Application Sheet V29

- All Application Sheets may be accessed from the OptiPrep™ Applications flash drive or from the following website: www.axis-shield-density-gradient-media.com, click on "Methodology" then "Viruses" to open up the Virus Index. Other OptiPrep™ Application Sheets on the preparation, harvesting and analysis of gradients may also be accessed from the top of the Index. These are:

    - Preparation of density gradient solutions: Application Sheet V01
    - Preparation of continuous and discontinuous gradients: Application Sheet V02
    - Preparation of self-generated gradients: Application Sheet V03
    - Harvesting gradients: Application Sheet V04
    - Analysis of gradients: Application Sheet V05
    - Concentration of virus samples: Application Sheet V06

1. Background to the use of OptiPrep™

In all comparative studies between CsCl and iodixanol, the recovery of virus infectivity is much higher and the particle:infectivity ratio much lower when viruses are purified in iodixanol. Although sucrose is generally less deleterious to viral infectivity than CsCl, it can nevertheless also have serious effects on certain important aspects of viral function; in particular the loss of surface glycoproteins from retroviruses has been noted [1]. This may be related to its viscosity, which is much higher than that of iodixanol solutions of the same density. Like CsCl, sucrose must be dialyzed before infectivity can be measured. In contrast many add-on techniques can be performed and cells infected with virus, without dialysis of iodixanol. The only analytical processing for which iodixanol must be removed is electron microscopy.

2. Gradient techniques

The first paper that was published on retrovirus purification in iodixanol gradients by Dettenhoffer and Yu [2] described the use of a sedimentation velocity gradient that spanned the range from 6-18% (w/v) iodixanol. The gradient was generated from multiple layers that differed in concentration from adjacent layers by only 1.2%. It is highly likely that the gradient becomes more or less a continuous one, even though the centrifugation is only for 1.5 h at 200,000 *g*. The gradient is very effective for separating the virus from smaller components and is a widely used format for studying virus assembly. Although a sedimentation velocity separation requires the sample volume to be kept a minimum, the strategy has been widely used and extended to the purification and fractionation of other viruses. This sedimentation velocity format has been used for Moloney murine leukaemia virus [3] and Mason-Pfizer monkey virus [4]. Although the gradient concentration range is invariant, the centrifugation times and *g*-force have generally been modulated downwards: 100,000 *g* for 1 h [3] and 164,000 *g* for 30 min [4]. Another important consideration in the use of a sedimentation velocity format is the low density at the top of the gradient; use of a high-density cushion to concentrate the virus renders layering of the virus on top of the gradient difficult.

Pre-formed buoyant density gradients are much less problematic regarding sample application, generally the iodixanol concentration at the top of the gradient is 10% (w/v) or higher and there is no obvious reason why the virus cannot be loaded in a dense solution at the bottom of a continuous gradient or made part of one of the denser steps of a discontinuous gradient, rather than layering on the top. Iodixanol gradients spanning the range 20-40% [5]; 10-30% [6], 10-32% [7] or 10-40% [8,9] are examples for retrovirus purification; *g*-forces are generally 100-150,000 *g* for 4-20 h; the exception being the much lower 35,000 *g* for 16 h used for Mason-Pfizer monkey virus [8]. There is no doubt that lower *g*-forces can be of benefit in maintaining functionality of many biological particles and this may also be true of viral particles. Discontinuous gradients have also been used for the purification of HIV virus-like particles [10,11]

Self-generated gradients are no doubt the easiest to prepare (the virus suspension is simply adjusted to a single median density) and offer no problems at all when considering a previous virus concentration step on to a high-density cushion. These gradients have been used for HIV-1 [12] HTLV-1 and HERV-H [13]. The method requires a vertical or near-vertical rotor and the centrifugation conditions are more or less standardized at approx. 350,000 *g* for approx. 3h.

There are also a few instances of the use of a simple barrier through which the HIV particles sediment [14,15], thus effecting a partially purification and concentration the virus. The barrier is usually approx. 8% (w/v) iodixanol and centrifugation is at 50,000 *g* for 1-2 h.

- ♦ In an interesting comparison of two methods of concentrating HIV-1 particles, pelleting through a 20% sucrose cushion or banding on to an OptiPrep™ cushion, Kol et al [16] observed that the sub-membrane Gag layer of the HIV isolated using OptiPrep™ was approx. 82% intact, while using sucrose it was only 60% intact; the mechanical properties of the virus were also adversely affected by sucrose.
- ♦ Meckes and Raab-Traub [17] also observed that the iodixanol-purified virus was free of exosomes, unlike that obtained in scrose gradients.

3. References (to Sections 1 and 2)

1. Palker, T.J. (1990) *Mapping of epitopes on human T-cell leukemia virus type 1 envelope glycoprotein* In: Human Retrovirology: HTLV (ed. Blattner, W.A.) Raven Press, NY, pp 435-445
2. Dettenhoffer, M. and Yu, X-F. (1999) *Highly purified human immunodeficiency virus type 1 reveals a virtual absence of Vif virions* J. Virol., **73**, 1460-1467
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4. Comprehensive retrovirus bibliography

- The references are divided alphabetically into virus type and where required they may be further separated (also alphabetically) according to the **research topic**. In all sections references are listed alphabetically by first author; multiple first author papers are listed chronologically. To aid selection key words in the titles are highlighted in light blue.

1. Foamy virus (Spumaviridae)

1-1. Capsid assembly/particle release

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1-3. Immune response

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1-5. Purification

Spannaus, R., Miller, C., Lindemann, D. and Bodem, J. (2017) *Purification of foamy viral particles* Virology **506**, 28–33

2. Human immunodeficiency virus-1 (HIV-1)

2-1. Actin

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2-3. Autophagy

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2-4. Budding

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2-8. Cholesterol (membrane)

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