OptiPrep™ Application Sheet V06

Concentration of viruses prior to gradient purification

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- To access other Application Sheets referred to in the text return to the Virus Index; key Ctrl "F" and type the V-Number in the Find Box.

1. Background

Often the volume of virus-containing fluid is too large to be processed on a density gradient without prior concentration. Therefore, after clarification of the virus suspension by low-speed centrifugation (2000-4000 *g* for 15-30 min) and maybe passage through a filter of pore size 0.2 or 0.45 μm; concentration from the suspension can be achieved by one of the following procedures.

2. Pelleting

The simplest method is to centrifuge the filtrate to pellet the virus. Although this could be executed in a fixed-angle rotor, the more compact pellet achievable with a swinging-bucket rotor may account for the widespread use of such rotors. The centrifugation conditions vary quite widely and will depend on the virus type; the g-force may be as low as 50,000 *g* (for 1.5 h) or as high as 160,000 *g* (for 1 h) and the time may be as long as 4 h (at 125,000 *g*). To avoid the loss of infectivity that occurs during pelleting, the lowest g-force and shortest time that allow maximum recovery of infectivity should be used. The virus pellet can be resuspended in a solution of any density for further purification.

If the virus-containing suspension is a cell lysate containing subcellular organelles, the initial clarification step will remove large debris and nuclei. A 0.2 μm filter will remove many of the larger organelles (mitochondria, lysosomes, peroxisomes etc). The subsequent virus pelleting conditions will co-sediment of most of the microsomal membrane vesicles, except perhaps at the very lowest g-forces and times (e.g. 50,000 *g* for 1.5 h), which may be insufficient to pellet the smallest vesicles.

It is not entirely clear whether the loss of infectivity that occurs during pelleting is due to the aggregation of the viral particles, the high hydrostatic pressure at the bottom of the tube or the shearing forces that are necessary to disperse the virus pellet after the centrifugation, or a combination of all three effects.

- In order to overcome the shearing force problems some workers have allowed pelleted Semliki Forest virus to disperse itself in a buffered saline solution at 4°C overnight.

3. Low-density barriers

A commonly used alternative to direct pelleting from the virus-containing fluid is the use of a low-density barrier through which the virus is pelleted. A variety of types of barrier have been used, which traditionally were 15-20% (w/v) sucrose or, occasionally, 30%. More recently, these have been replaced with 5-15% (w/v) iodixanol barriers and since an iodixanol gradient is used in the subsequent purification, then it makes for good practice to expose the virus to just one type of gradient solute. The g-forces and centrifugation times are similar to those used in direct pelleting, i.e. generally 50,000-160,000 *g* for 1-2 h. This barrier technique will allow some preliminary purification from soluble proteins and from small low-density vesicles. The same considerations regarding the resuspension of the pellet apply here as to simple pelleting as described in Section 2. The virus pellet can be resuspended in a solution of any density for further purification.

4. High-density barriers

The use of a cushion of dense medium, whose density is greater than that of the virus itself, will allow the virus to be concentrated at the interface of the cushion and so avoid the harsh conditions associated with pelleting. Commonly the cushion is either 50% (w/v) iodixanol or pure OptiPrep™.

This strategy is well established as one that will maximize the recovery of infectivity and should be regarded as the ideal way of concentrating virus.

4a. Cushion volume considerations

For self-generated gradients

The virus banding is invariably carried out in a swinging-bucket rotor in order to minimize the volume of cushion that is required. In 14 ml tubes (e.g. for the Beckman SW41Ti) a cushion of approx 1 ml might be regarded suitable, and in a larger 38 ml tube (e.g. for the Beckman SW28) a cushion of 2-3 ml might be better. The methodology is particularly suitable to subsequent purification in a self-generated gradient; see the following examples of this technique:

- Application Sheet V08, Herpes virus
- Application Sheet V31, human endogenous retrovirus (HERV-H) and human T-cell lymphotropic virus (HTLV-1)
- Application Sheet V25, Ebola virus
- Application Sheet V18, Norwalk virus
- Application Sheet V20, Hepatitis C virus
- Application Sheet V21, SARS-Coronavirus

A self-generated gradient strategy does not require the virus to be applied to a gradient in a small volume, but instead involves filling the centrifuge tube with the virus in a solution of uniform density (normally 20-25%, w/v, iodixanol). Thus, for example, after banding the virus on a 2 ml 50% (w/v) cushion, all of the supernatant (except for 2 ml) can be removed and mixing the residual contents of the tube will yield the required suspension of virus in 25% iodixanol.

For pre-formed gradients

If the virus is to be loaded on top of a pre-formed continuous or discontinuous gradient then there are some limitations to the cushion-banding technique that need to be considered, if for example the iodixanol concentration at the top of the subsequent gradient is, for example 6% (w/v). Even with just 0.5 ml of a 50% (w/v) iodixanol cushion, mixing the residual contents of the tube after removal of the majority of the supernatant would require at least 5 ml of supernatant to reduce the iodixanol concentration to <5% iodixanol. In this situation therefore it is important to remove as much as the cushion as possible before harvesting the virus band.

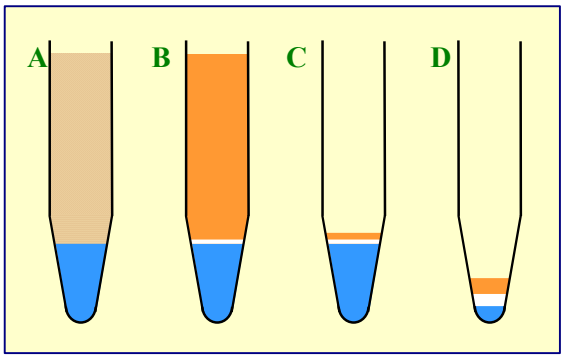

Figure 1: Virus banding on dense iodixanol cushion in a Beckman ***k***onical tube. A: Before centrifugation; B: After centrifugation; C: After removal of most of supernatant; D: After removal of most of cushion

Conical tubes facilitate this process, and Beckman manufacture ***k***onical™ tubes for all their swinging-bucket rotors. Small volumes of cushion occupy a greater linear height in a conical tube than in the traditional round-bottomed ultracentrifuge tube and most of this cushion can also be more easily removed prior to harvesting the virus band in a small volume of supernatant. A thin metal cannula or length of Teflon tubing, attached to a syringe may be used to remove as much of the cushion as possible after centrifugation. Figure 1 summarizes the sequence of events. It should then be possible to remove the virus band in the residual supernatant, whilst aspirating as little of the cushion as possible.

Coleman et al [1] only used 0.22 ml of cushion and removed all of the supernatant (except for the last 0.22 ml) and then harvested all of the remaining liquid (including the cushion) and diluted the suspension 2.5x with buffer before centrifuging it at 6000 *g* for 24 h at 4°C to pellet HIV-1. Since the final volume of suspension was very small, it was possible to pellet the virus efficiently at this very

gentle g-force. This would not have been feasible with a large volume of virus suspension, because of the longer sedimentation path length and low g-force at the top of the sample.

If the concentrated virus is to be loaded in a dense solution beneath a pre-formed gradient for a separation on the basis of its buoyant density then the need to eliminate as much of the cushion as possible does not apply.

5. References

1. Coleman J.E., Huentelman, M.J., Kasparov, S., Metcalfe, B.L., Paton, J.F.R., Katovich, M.J., Semple-Rowland, S.L. and Raizada, M.K. (2003) *Efficient large scale production and concentration of HIV-1-based lentiviral vectors for use in vivo* Physiol. Genomics, **12**, 221-228

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