PURIFICATION OF VIRAL VECTORS FOR CELL TRANSDUCTION IN CANCER STUDIES

- This Axis-Shield Mini-Review brings together information on the purification of all the principal viral vectors for the transduction of cancer cells; it covers rAAV, parvovirus, adenovirus and Herpes virus. It summarizes the OptiPrep™ methodology (Section 1) used in the purification of these vectors and includes a comprehensive reference list of papers (Section 2) reporting the use the vectors in cancer studies.

1. Technical background to the use of OptiPrep™
1a. Comparison with other density gradient media

Compared to CsCl and sucrose there are procedural advantages to the use of OptiPrep™

- OptiPrep™ is a sterile solution of 60% (w/v) iodixanol; it is simply diluted with saline to prepare sterile gradient solutions. It is the only gradient medium manufactured under strict FDA and EU cGMP compliance.
- CsCl and sucrose are both toxic to cells.
- Iodixanol is non-toxic to cells; it has very low endotoxin levels (<1 EU/ml); measured levels on each batch are usually <0.13 EU/ml.
- CsCl must be removed prior to HPLC or gel electrophoresis; iodixanol rarely needs removing prior to further processing, except for electron microscopy studies.
- CsCl gradients lead to major reductions in viral infectivity. Virus from iodixanol gradients shows a higher % recovery of infectivity and much lower average particle/infectivity ratios compared to that from CsCl gradients (see Table 1)
- Although sucrose is generally less deleterious to viral infectivity than CsCl, it can nevertheless have serious effects on viral structure; in particular the loss of surface glycoproteins from enveloped viruses [1].

Table 1 Comparison of OptiPrep™ and CsCl for purification of rAAV; data from Hermens et al (ref 2)

<table>
<thead>
<tr>
<th>Pre-gradient treatment</th>
<th>Gradient medium</th>
<th>% Recovery</th>
<th>Particle/infectivity ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium sulphate</td>
<td>CsCl</td>
<td>0.1-60</td>
<td>250 - 100,000,000</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>OptiPrep™</td>
<td>26-120</td>
<td>264 - 1333</td>
</tr>
</tbody>
</table>

1b. Purification of rAAV and parvovirus vectors

There are two methods (see Figure 1) for the purification of rAAV vectors and each has its own advantages. The discontinuous gradient (Figure 1a) was designed for a 39 ml fixed-angle rotor. Large-volume swinging-bucket rotors are not normally capable of achieving 350,000 g, with such a rotor the centrifugation time would need to be increased. Any soluble proteins band well away from the rAAV [3]. The NaCl in the 15% (w/v) iodixanol minimises any association between the rAAV and these proteins.

The continuous gradient (Figure 1b) was designed for a near-vertical rotor and it has the great merit of ease of setting up, but the rAAV may be less well resolved from low MWt soluble proteins. The lack of any interfaces will minimise any particulate aggregation [2].
Parvovirus vectors have generally been purified using the discontinuous iodixanol gradient method.

1c. Purification of adenovirus vectors
In 2005 the discontinuous iodixanol gradient described in Figure 1a was first reported for the purification of adenovirus vectors by Manninen et al [4]. The gradients are centrifuged in an approx. 12 ml swinging-bucket rotor at 100,000 g for 6 h. Later Arpiainen et al [5] used a modified gradient of 15%, 30% and 40% (w/v) iodixanol with centrifugation at 100,000 g for 14-16 h.

1d. Purification of herpes virus vectors
Herpes simplex virus (HSV) was the first virus to be purified in self-generated gradients of iodixanol and it is now a very widely used method for HSV vectors. Self-generated gradients make sample handling very easy; the crude virus suspension is adjusted to approx. 25% (w/v) iodixanol by addition of OptiPrep™; transferred to a tube for a vertical or near-vertical rotor and centrifuged at approx. 350,000 g for 1.5-2.5 h. During this time the gradient forms and the virus moves to its banding density. As with the continuous gradient method for rAAV (see Section 1b, above) there are no interfaces to produce particulate aggregation. The shape of the density profile changes gradually from 1 to approx. 4.5 h, after which the profile is more or less stable. Figure 2 compares infectivity and density profiles after centrifugation of a suspension of HSV in 25% (w/v) iodixanol in a Beckman VTi65.1 vertical rotor after centrifugation at 350,000 g for 1.5 and 2.5 h. The shallower S-shaped gradient formed after 1.5 h allows a better separation of the lighter immature virus.

If HSV (or any virus) is first concentrated by sedimentation on to a 50% (w/v) iodixanol cushion, the subsequent processing for a self-generated gradient is simple; while if the virus is layered on top of a pre-formed gradient, reducing the iodixanol concentration of the virus suspension to allow this can pose some serious problems.

1e. Axis-Shield OptiPrep™ Application Sheets
Detailed protocols for the isolation of the viral vectors described above may be accessed from the Index of the “Viruses” file either on the Axis-Shield OptiPrep™ Applications CD or from the following Axis-Shield website: www.axis-shield-density-gradient-media.com. Other relevant OptiPrep™ Application Sheets may also be accessed from the top of the Index.
• Recombinant adeno-associated virus and parvovirus
• Adenovirus
• Herpes virus – self-generated gradient
• Herpes virus – pre-formed gradients
• Preparation of density gradient solutions
• Preparation of continuous and discontinuous gradients
• Preparation of self-generated gradients
• Harvesting gradients
• Analysis of gradients
• Concentration of virus samples

2. Bibliography
2a. Research topics
rAAV vectors in anti-angiogenesis
rAAV vectors in anti-viral activity
rAAV vectors in transduction of glial/glioma/glioblastoma cells
rAAV vectors in leukaemia
rAAV vectors, effect on tumour cells
Parvovirus vectors in tumour cell transduction
Adenovirus vectors
HSV vectors in anti-angiogenesis
HSV as helper virus
HSV vector construction and delivery
HSV vector delivery to glioblastoma
HSV vector in Kaposi sarcoma

2b. References


