OptiPrep™ Application Sheet V37

Purification of viruses from non-mammalian cells and tissues

- 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 **2020Virapp** file and select the appropriate **V number**
- This Application Sheet describes principally the isolation of viruses (*Phycodnaviridae)* from algal cells (Section 1), but also viruses from protozoa (Section 2), nematodes (Section 3), marine arthropods (Section 4), plant cells (Section 5) and yeast (Section 6).
- Note that **Application Sheet V13** describes the purification of the Group I iridovirus isolated from an aquatic species (Singapore grouper fish)
- Note that **Application Sheet V38** describes the purification of bacteriophages

1. Isolation of viruses from algal sources (*Phycodnaviridae)*

Lawrence and Steward [1], recommended making all iodixanol gradient solutions by dilution of OptiPrep™ with the regular culture medium. This will maintain the normal osmolality of the solutions. This approach has been adopted by many authors.

Moniruzamann et al [2] purified the **Megaviridae virus *Aureococcus anophagefferns*** (trivial name "brown-tide virus") using a discontinuous gradient of 2.6 ml each of 25%, 30%, 35% and 40% (w/v) iodixanol, on to which 1.5 ml of sample was layered and centrifuged at 185,000 *g* for 14 h. Certainly during this time the gradient would have become more or less continuous. Lawrence and Steward [1] recommended the use of a 4 ml gradient (total volume) with 1 ml of sample at approx. for 200,000 *g*, for 4.25 h, or in a scaled-up version (13 ml tubes in a Beckman SW41 rotor) at approx. the same *g*-force for 7.25 h. The authors also noted that for most downstream techniques (except electron microscopy) it was not necessary to remove the iodixanol and Malitsky et al [3] observed that the iodixanol gradient maintained the virus morphology and infectivity.

Essentially the same gradient and centrifugation conditions were used by Rosenwasser et al [4] and Schatz et al [5] for the purification of ***Emiliania huxley* virus**.

***Paramecium bursaria* Chlorella virus** 1 (a "Giant" virus) was treated with 1% (v/v) NP-40 before sedimenting and resuspension in 50 mM Tris-HCl, pH 7.8 before initial loading on to a 10-40% (w/v) sucrose gradient and centrifugation for 20 min at 48,000 g_{av}. The recovered banded virus was treated with proteinase K to remove contaminating proteins before being centrifuged into a 20-40% (w/v) iodixanol gradient at 48,000 g_{av}, for 4h [6]. Dunigan et al [7] reported the same methodology and that the banding density of the virus was 1.178 g/ml (equivalent to approx. 33% (w/v) iodixanol. Working with the ***Acanthocytosis turfacea* Chlorella virus**, Petro et al [8] replaced the sucrose gradient with an iodixanol gradient and after treating with proteinase K to remove extraneous protein, treated the virus to a third round of iodixanol gradient centrifugation.

2. Isolation from protozoa (amoeba)

Faustovirus (*Vermamoeba vermiformis*) is an unusually dense virus, probably because it has two protein shells and the iodixanol gradient used to purify it is correspondingly dense [9]: the top-loaded 12 ml 40-60% (w/v) iodixanol gradient was centrifuged at 100,000 *g* for 24 h. The gradient effectively separated the intact virus from empty virions.

3. Orsay virus

Orsay virus infects nematodes (***Caenorhabditis elegans* or *Caenorhabditis briggsae***): it has unusually been purified in three stages [10]. In the first stage the virus in a buffered saline medium containing 2-mercaptoethanol and Triton X-100 was sedimented through a four step discontinuous

iodixanol gradient, comprising 15%, 25%, 32.5% and 40% (w/v) iodixanol at 150,000 *g* for 3 h. The iodixanol solutions contained a routine buffered saline containing EDTA and 2-mercaptoethanol; the 15% layer however contained 1 M NaCl. In this respect the method is very similar to that used for the purification of recombinant adeno-associated virus (rAAV) devised by Zolotukhin et al [11]; the high salt is present to minimize aggregation of the virus with soluble proteins at the first two interfaces. While the gradients are very similar, the centrifugation conditions are rather different: 150,000 *g* for 3 h for the Orsay virus and 350,000 *g* for 1 h for the rAAV. The former conditions used by Jiang et al [10] are likely to reduce any aggregation to a minimum.

The iodixanol in the harvested virus was then removed by ultrafiltration; the suspension treated with 1.0% Triton X100 and applied to a second gradient (25-45% w/v iodixanol), which also contained EDTA and 2-mercaptoethanol, and centrifuged at 150,000 g_{av} for 16 h. After harvesting the virus, it was adjusted to 40% (w/v) iodixanol and re-centrifuged in a near-vertical rotor (Beckman NVT90) at 250,000 *g* for 1.5 h. This step uses the ability of iodixanol to form self-generated gradients. It is important to use either a near-vertical or a vertical rotor to allow the formation of gradient with a useful density profile. Some small volume fixed-angle rotors can be used in place of the near-vertical rotor.

- For more information on the formation of self-generated gradients, using iodixanol, see **Application Sheet V03**.
- The authors [10] commented that iodixanol gradients permitted the recovery of virus of much higher purity, compared to that from CsCl-purified gradients. Moreover it permitted the detection of particles containing a minor post-translational modified variant of the fusion protein

4. From marine arthropods

White spot syndrome virus (WSSV) is a major pathogen for marine crustaceans; it has been isolated from ***Penaeus vannamei*** by Dantas-Lima et al [12]. After maceration of the shrimps in a buffered saline, large contaminants were removed from the suspension (3000 *g* for 20 min) the suspension (60 ml) was underlaid with a 10 ml cushion of 50% (w/v) iodixanol and centrifuged at 60,000 *g* for 2 h to concentrate the virus at the interface. After removal of the bottom 7.5 ml of cushion (this is best achieved by using a syringe attached to a long flat-tipped metal filling cannula, i.d. approx 1mm – see **Application Sheet V02** for more details). The bottom 5 ml of the remaining material is then recovered (i.e 2.5 ml of 50% idixanol + virus band + 2.5 ml of maceration medium) and layered under a discontinuous iodixanol gradient of 5%, 10%, 15% and 20% (w/v) iodixanol and centrifuged at 80,000 *g* for 3 h. The virus banding pattern depended on whether the original harvest was from tissues or haemolymph. For more information on this see ref 12.

- The authors commented on the very high purity and infectivity of the virus recovered from iodixanol gradients compared to that from CsCl gradients

- **5. From plant cells**
- Because of the relative paucity of information on plant viruses this section summarizes information from papers reporting the use of both Nycodenz® and OptiPrep®. Section 5-1 is concerned with the isolation of plant viruses using Nycodenz® gradients. As far as we know only red clover necrotic mosaic virus has been purified in iodixanol gradients (Section 5-2-2). The latter has been used more widely however for isolation of animal viruses grown in plant cells (these methods are summarized in Section 5-2-1).

- **5-1 Isolation of plant viruses in Nycodenz®**

One of the earliest papers that reported the use of Nycodenz® was that by Gugerli [13] who studied the banding of a number of plant viruses: various luteoviruses, tymoviruses, nepoviruses, tobamoviruses (including tobacco mosaic virus) and hodeiviruses. Gradients in the range 30-60% (w/v) Nycodenz® were used and the viruses generally had densities in the range 1.23-1.28 g/ml and significant advantages over the use of CsCl were noted (1.23 g/ml is approx equivalent to 42% Nycodenz®). Habili et al [14] reported the use the same gradients for a luteovirus (barley yellow dwarf

virus).

Cowpea mosaic virus consists of two separately encapsidated RNA molecules which, along with empty capsids, can also be fractionated on these 30-60% Nycodenz® gradients at approx 160,000 *g* for 15 h at 15°C.. The empty capsids band at a low density, while the other two particles are usually termed "middle" and "bottom" [15-18], reflecting their position in the gradient.

5-2 OptiPrep™-based methods

5-2-1 Virus-like particles propagated in plant cells

Blue-tongue virus-like particles (BTVLP) have been propagated in ***Nicotiania benthamiana*** using a plant expression system. The harvesting procedure involved the use of a 20-50% (w/v) iodixanol gradient generated from 3 ml each of four solutions (10% increment), which was overlaid by 24 ml of leaf extract and centrifuged at 85,000 g_{max} for 3h. The virus banded in the 30-40% iodixanol zone [19]. Brillault et al [20] used a very similar gradient. Van Zyl et al [21] first concentrated the BTVLP on to a 40% iodixanol cushion (79,000 *g* for 2 h) then analyzed it on a discontinuous iodixanol gradient (20-60%) under the same centrifugation conditions. Similar gradients were used for purifying polyoma VLPs, also propagated in ***Nicotiania benthamiana*** leaves [22].

HIV virus-like particles (VLPs), also grown in ***Nicotiania benthamiana***, were layered on top of a 10%, 20%, 30%, 40%, 50%, 60% (w/v) iodixanol gradient and centrifuged 210,000 *g* for 4 h. The VLPs banded around the original position of the 10%-20% interface [23, 24]. Human papillomavirus pseudovirions have also been grown in ***Nicotiania benthamiana***. Larger particles from the plant extract were first removed by low speed centrifugation (10,000 *g* for 15 min) and concentrated in a two layer (30% - 50%) sucrose gradient at approx. 110,000 *g* for 75 min. After recovery of the VLPs and dialysis to remove the sucrose, they were layered atop a 20%, 33%, 40%, 50% (w/v) iodixanol gradient and centrifuged at approx. 110,000 *g* for 4h. The VLPs banded around 33% iodixanol [25].

- A review of plant-grown viruses and VLPs has recently been published [26].

- **5-2-2 Red clover necrotic mosaic virus (RCNMV)**

Lockney et al [27, 28] studied the potential for RCNMV capsid to be used in specific cell targeting in the delivery of therapeutic reagents. The capsid was labeled with a fluorescent peptide but the authors gave rather little detailed information about the purification procedure. A generic 0-54% (w/v) iodixanol gradient (containing 20 mM Tris-HCl, pH 8.0, 120 mM NaCl, 1 mM EDTA) centrifuged at 175,000 *g* for 2 h may be a good starting point. The fluorescent-marked particles banded about two-thirds the way down the gradient. It may be necessary to modulate the centrifugation time (or speed).

6 Yeast retrotransposons

Tf1 retrotransposons are being studied as important models for the activity of retroviruses. They have similar structures and propagation mechanisms. Kim et al [29] used the discontinuous iodixanol gradient method that is widely used for the purification of rAAV (see **Application Sheet V14**). Various Gag species were identified in the denser regions of the gradient that were assembled into virus-like particles (VLPs); these were well separated from the lighter ones that did not assembles into such particles [29]. Ty1 retrotransposons in VLPs were resolved in a discontinuous 5-50% (w/v) iodixanol gradient centrifuged at approx 200,000 *g* for 3h [30]

7 References

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