# OptiPrep™ Application Sheet V26

## Purification of Group V ((-)ss) RNA viruses: *Orthomyxoviridae*

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- This Application Sheet covers the purification of influenza A virus
- **To access other Application Sheets** referred to in the text: return to the **2020Virapp** file and select the appropriate **V number.**
- Section 6 is devoted to the purification of virus-like particles

**1. Background**

The *Orthomyxoviridae* family comprises influenza virus A, B and C, Isavirus and Thogotovirus. All of the published papers reporting the use of iodixanol gradients for the purification and analysis of *Orthomyxoviridae* viruses are concerned with influenza virus A. It is highly likely that, because of the basic overall similarity in structure, the methods described below would be suitable for any members of this family.

There are now many published papers that report the use of iodixanol gradients not only to purify viruses but also to investigate their assembly. 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, in solutions of the same density, is much higher than that of iodixanol.

Like CsCl, sucrose must be dialyzed before infectivity can be measured. In contrast both infectivity measurements using cultured cells and many add-on techniques can be performed without dialysis of iodixanol. Combined with the availability of OptiPrep™ as a sterile solution, this makes the use of OptiPrep™ for virus purification and assembly analysis much more convenient than the use of either CsCl or sucrose.

**2. Solutions required**

**A.** OptiPrep™ (60% w/v iodixanol)

**B.** OptiPrep™ diluent: any suitable buffered saline such as PBS is suitable or a buffer such as 100 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4 [2,3]. As long as the solution is isoosmotic, then all iodixanol solutions produced from OptiPrep™ will also be isoosmotic. **For more information on the production of gradient solutions see OptiPrep™ Application Sheet V01**.

**3. Rotor requirements**

A routine swinging-bucket rotor capable of approx. 100,000 *g* (e.g. Beckman SW28 or SW41Ti)

**4. Protocol** (carry out all operations at 4°C)

**4a. Clarification and concentration of virus fluid**

The virus is normally grown in a cell culture (often MDCK cells) although Chou et al [3] used the allantoic fluid of chicken eggs. An initial clarification of the virus-containing isolate to remove cells and larger debris is carried out usually between 400 *g* and 800 *g* for 5-15 min [4-6] although much higher speeds are sometimes used such as 2600 *g* for 5 min [2] or in the case of allantoic fluid 3000 *g* for 30 min [3].

The virus is usually, but not always [6] concentrated prior to gradient purification. Sometimes the virus is pelleted through a 20% (w/v) sucrose cushion at approx. 100,000 *g* for 2h [2,3] or a 14% (w/v) iodixanol cushion for 1 h at 55,000 *g* [4] or 88,000 *g* [5]. The higher *g*-force and time necessary

for the sucrose cushion reflect the higher viscosity of this medium. Since the virus is finally purified in an iodixanol gradient it may be less stressful for the virus to use the same medium for the cushion.

**4b. Gradient purification**

Continuous iodixanol gradients have often been used for the final purification step. The following conditions have been used: 10-30% (w/v) iodixanol at approx 100-120,000 *g* for 3 h [2,3], 14-26% (w/v) at 55,000 *g* for 45 min [5] and two sequential gradient centrifugations through 10-26% (w/v) iodixanol at 55,000 *g* for 45 min [6,7]. The short time and low *g*-forces used in the latter two examples may suggest that the higher *g*-forces and longer times more frequently reported for this and other viruses may be unnecessary. More recently Hutchinson et al [8] used a 10-40% (w/v) iodixanol gradient following concentration through a 10% iodixanol cushion. In an interesting adaptation by Thompson et al [9] the sample was adjusted to 20% (w/v) iodixanol before fractionation on 25.5-40% (w/v) iodixanol gradient at 350,000 *g* for 6h. The raised density of the sample layer would minimize any aggregation at the gradient interface. This study also investigated the resolution from baculovirus.

Discontinuous gradients of 10%, 15%, 20%, 25% and 30% (w/v) iodixanol at, 100, 000 *g* for 3 h have also been used [10]; the virus banded at the 15/20 boundary was the most pure, although virus was present at the two adjacent boundaries.

A detailed comparison of the various iodixanol methodologies has not been carried out. It is worth pointing out however that because of the generally lower density of viruses in iodixanol gradients compared to sucrose; the consequent lower viscosity of iodixanol gradients may allow the use of more mild centrifugation conditions.

**5. Comments**

Shaw et al [2] compared the banding of influenza virus in sucrose and iodixanol gradients. Firstly to band the virus it was necessary to use a 30-60% sucrose gradient, which covers a density range of 1.12 to 1.28 g/ml; while with the range of a 10-30% iodixanol gradient was 1.058-1.16 g/ml. This reflects the lower density of the virus in an isoosmotic iodixanol gradient compared that of a hyperosmotic sucrose gradient. In the latter the virus has a median density of approx 1.20 g/ml, while in iodixanol it was approx 1.14 g/ml. Another serious consequence of the hyperosmotic nature of sucrose gradients is that all osmotically active particles, such as membrane vesicles, also lose water and approach a limiting density as they move through the gradient. So the sucrose gradients were unable to resolve the CD9 protein of the virus from the MHC-1 marker for exosomes; while the density of the latter in the iodixanol gradient (approx 1.10 g/ml) was much lower than that of the virus. The gradients described by Latham and Galarza for virus-like particles (see Section 6) have also been used by LeBouder et al [11] for influenza virus (2h at 80,000 *g*). A self-generated iodixanol gradient (starting concentration 18% (w/v) iodixanol) centrifuged in a vertical rotor at 350,000 *g* for 6 h has also been used [12].

**6. Virus-like particles (VLPs)**

The purification of VLPs in iodixanol gradients was first described by Latham and Galarza [13]. After pelleting from a clarified culture medium the VLPs were pelleted at 200,000 *g* and loaded on to a 14-60% (w/v) iodixanol gradient centrifuged at 200,000 3 *g* for 3.5 h. Similar centrifugation conditions were reported in refs 14-16, but Sulli et al [17] used a shallower gradient of 10-30% (w/v) iodixanol. An identical gradient was used by Chlanda et al [18] centrifuged at 200,000 *g* for 2 h after an initial concentration by pelleting through a 30% sucrose cushion. The purification and use of VLPs has recently been reviewed [19].

- **For information on preparation of continuous gradients see OptiPrep™ Application Sheet V02**
- **For information on harvesting of gradients see OptiPrep™ Application Sheet V04**
- **For information on gradient analysis see OptiPrep™ Application Sheet V05**
- **For more information on virus concentration see OptiPrep™ Application Sheet V06**

## 7. References


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Serumwerk Bernburg AG • Hallesche Landstraße 105 b • 06406 Bernburg • Germany
phone: +49 3471 860 429 • diagnostic.serumwerk.com • diagnostic@serumwwerk.com • serumwerk.de