OptiPrep™ Application Sheet V36

Purification and analysis of Group VII (ds RNA-RT): *Hepadnaviridae*: *Orthohepadnavirus:* hepatitis B virus

- 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**
- For recent reports of the use of iodixanol gradients see Section 3

1. Background

The two genera of the *Hepadnaviridae* family are *Orthohepadnavirus* and *Avihepadnavirus* which are represented by hepatitis B virus and duck hepatitis B virus respectively, both of which have been purified and analyzed using iodixanol gradients.

In all comparative studies between CsCl and iodixanol, it has been shown that 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 enveloped viruses has been noted [1]. This may be related to its viscosity, which is much higher than that of iodixanol. 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.

2. Methodology and results

Prior to the development of an OptiPrep™ based method, Kock et al [2] had used a 10-50% (w/v) Nycodenz gradient (the solid Nycodenz was dissolved in an isoosmotic solution of 140 mM NaCl, 1.5 mM $MgCl_2$, 50 mM Tris-HCl, pH 8.0 containing 0.5% NP40). Centrifugation was carried out at approx. 250,000 g_{max} for 40 min at 20°C (in a Beckman TLS55 swinging-bucket rotor). The gradient showed that the viral DNA sedimented with the core protein of duck hepatitis B virus. The method was adapted to a discontinuous gradient of iodixanol (10% increments) using the same centrifugation conditions and applied to human hepatitis B virus grown in mammalian cultured cells [3,4]. The density range would have been almost identical in the two cases, but, whilst the denser regions of the Nycodenz gradient would have been hyperosmotic, the entire iodixanol gradient would have been more or less isoosmotic. Whether this has any influence on the banding density of the virus is not clear.

The 10-50% (w/v) iodixanol gradient effectively demonstrated that an HBV Pol-interacting host factor (DDX3) was incorporated into the nucleocapsid and that this was dependent on HBV-Pol. The core proteins demonstrated a peak banding around three-quarters of the way down the gradient. This was equivalent to approx. 35% (w/v) iodixanol or approx. 1.19 g/ml. The same group also showed the incorporation of Pol into nucleocapsid was proteinase K-resistant.

Bardens et al [5] used a very different gradient format, the cells were Dounce homogenized in a hypotonic medium containing 1 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.5 (no detergent) and after an initial low speed centrifugation to remove cell debris the supernatant was adjusted to 40% (w/v) iodixanol and overlaid by a solution of 28% (w/v) iodixanol and centrifuged at 100,000 *g* for 3h. The virus particles presumably banded around the interface between the two iodixanol solutions.

The only paper reporting the analysis of duck HBV was concerned with the inhibition of HBV replication [6]. The cells were homogenized in a routine medium often used in membrane fractionation studies (0.25 M sucrose, 1 mM EDTA, 60 mM HEPES-NaOH, pH 7.4). Any un-homogenized cells and partially disrupted cells were sedimented at 100 *g* for 4 min; the pellets were re-homogenized in the

same medium after being washed twice; all of the supernatants were bulked together. A post-nuclear + heavy mitochondria supernatant was prepared at 2,500 *g* for 10 min (the pellet was washed and the supernatants combined). This supernatant was loaded on to a discontinuous gradient of 5, 10, 15, 20, 25 and 30% (w/v) iodixanol. These solutions were prepared from dilutions of OptiPrep™ with the homogenization buffer (**see Application Sheet V01 for more information about the preparation of gradient solutions**). The gradients were centrifuged at 288,000 *g* for 2 h (Beckman SW41Ti rotor). They were used to investigate the effects of treatment of cells with a cationic peptide Deca-$(arg)_8$, which has been shown have significant anti-viral activity. Abdul et al [6] demonstrated that the DNA, nucleocapsid and preS/S proteins banded quite broadly towards the bottom of the gradient (ρ = 1.14/1.22 g/ml), although some capsids and preS/S banded at a slightly lower density. Deca-$(arg)_8$ treatment caused a marked shift of the DNA and core protein to a lower and more sharply-defined density while the preS/S proteins shifted to a similarly well-defined higher density. The gradient thus seems to offer a high-resolution analysis of these macromolecules.

3. Recent published papers

Komatsu et al [7] used a discontinuous iodixanol gradient in a small volume fixed-angle rotor (6%, 10%, 20%, 30%, 40%, 50% (w/v), i.e covering a similar density range to that described in refs 3 and 4. The authors used a small-volume high performance rotor (probably the Beckman TLA-110 or similar) for 4 h at approx 400,000 g_{av} to purify the virus from patient specimens. The gradient would undoubtedly become continuous (due to diffusion and self-generation) during this time, but whether these centrifugation conditions would allow a discrimination of enveloped and non-enveloped virions is not clear. Verrier et al [8] also investigated the virus isolated from patient specimens in a 10-45% (w/v) iodixanol gradient but did not provide any further experimental detail. Virus was also isolated both from HepG2215 cells and patient plasma [9]. Li et al [10] refer to a method first used by Feng et al [11] for hepatitis A virus which involves the use of an 8-40% (w/v) iodixanol gradient, centrifuged at 141,000 *g* for 48 h. It is not clear if the virus banding was similar or different. In the studies by Lam et al [12] the hepatitis B virus that was expressed into the culture fluid from HepaRG cells was analyzed on an 18-50% iodixanol gradient centrifuged at 134,000 *g* for 2 h.

4. References

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