Purification and analysis of papillomaviruses

♦ OptiPrep™ is a sterile 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
♦ This Mini-Review principally provides (in Section 2) a bibliography of all those papers reporting the use of OptiPrep™ in the purification and analysis of papilloma virus and virus-like particles. Section 1 briefly summarizes the advantages of using OptiPrep™; the gradient strategy used for papillomavirus and the technical data that is available.

1. Technical background to the use of OptiPrep™

1a. Background
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 enveloped viruses, particularly 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. Most iodixanol gradients can also be made isoosmotic over the entire density range.

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. The only analytical technique for which removal of the iodixanol is essential is electron microscopy. Consequently iodixanol is being increasingly used for the purification of papillomavirus particles from lysed cultured cells.

1b. Gradient strategy
Buck et al [2] observed that papillomavirus vector stocks could be purified by an iodixanol gradient centrifugation procedure that was substantially more effective than the standard CsCl gradient purification. The methodology developed [2] has been used more or less unchanged in all subsequent studies and has been found to be effective for human, bovine and cotton-tail rabbit viruses. A continuous gradient is usually generated from equal volumes of 27%, 33% and 39% (w/v) iodixanol allowed to diffuse for 3-4 h at room temperature, after which the clarified sample is laid on top. Although the continuous gradient might be prepared from 27% and 39% iodixanol using a two-chamber gradient maker, use of the latter is technically more difficult with these small volume gradients. The separation of the L1 protein from both DNA-containing and empty virus-like particles after 234,000 g for 3.5 h at 16°C is considered to be part buoyant density and part sedimentation velocity [2], so top-loading of the sample cannot be replaced by other frequently-used strategies such as bottom-loading of the sample or the use of self-generated gradients.

1c. References (to Sections 1a and 1b)

♦ The gradient protocol for the purification of isolation of papillomavirus particles (Application Sheet V10) may be accessed from the OptiPrep™ Applications flash-drive or from the following website: www.axis-shield-density-gradient-media.com, click on “Methodology” then “Viruses” to open up the Virus Index. Other OptiPrep™ Application Sheets on the preparation and harvesting of gradients may also be accessed from the top of the Index.
2. Bibliography

- This bibliography provides a comprehensive reference list of all the papers reporting the use of OptiPrep™ for papillomavirus purification, published before the end of January 2017.
- The references are divided alphabetically into “Research topic” sections and subsections.
- All references are listed alphabetically according to First Author.
- The vast majority of published papers describe studies on human papillomavirus (not flagged); those studies on bovine (B), canine (C), cotton-tailed rabbit (CTR), equine (E), Macaques (MQ) and mouse (M) are indicated as shown. In many cases these flagged papers will also include work on the human virus.

B-cell anergy reversal

Baculoviral vectors

Capsids and capsid protein
Capsid assembly

DNA, separation from

Dynein interacting domains

L1 capsid protein

Maturation
Neutralization-sensitive epitopes


Tumour cell binding


Cell entry/targeting

Autophagy inhibition


Clathrin/caveolin


Cyclophilin receptors


Cysteine proteases


Dynamin inhibition

Abban, C.Y., Bradbury, N.A. and Meneses, P.I. (2008) HPV16 and BPV1 infection can be blocked by the dynamin inhibitor dynasore Am. J. Therapeut., 15, 304-311 (B)

Dynein light chain requirement


Focal adhesion kinase activation


Heparan sulphate receptors


Inhibition by thio-reactive agents

Laminin-5 binding

L1/L2 protein interactions

Microtubules, L2 interaction with

Phosphatidylinositol-3 kinase

PML expression

γ-Secretase requirement

Tetraspannin-enriched domains

Epithelial cell, expression in

Gene expression

Genome
Amplification

Encapsidation
Packaging

Immunogenicity

Infection
Anti-L1 antibodies

Antivirals

Cell cycle progression requirement

L2 Cysteine residues

Heparan sulphate

Integrins

Optical imaging
Restriction factors

γ-Secretase requirement

Single particle tracking

Skin/vaginal lesions

Syndecan-1

Infection inhibition
Carrageen

Cholesterol derivatives

α-Defensins
E. coli sulphated polysaccharides

Genome, nuclear transport block

Heparan sulphate binding

High affinity ligands

L1/L2 antibody, comparison of

Mucins

Infectious virus production
Genome incorporation

Vacular ATPase

Intracellular assembly
L1/L2 proteins

L2 cysteine residues
Redox gradient dependence

Intracellular trafficking:
Cysteine proteases

Dynein interacting domain

Endosomal trafficking

Genome, nuclear transport block

Inhibition by thio-reactive agents

L2 capsid protein


PML expression

Polyethylenimines

Keratinocyte/expression in/interactions


Langerhans cell activation

Methodology

Neutralization assays

Neutralizing antibodies
Capsid protein
L1 epitopes
L2 epitopes:

Oropharyngeal cancer
Ovarian cancer therapy

Plants, production in

Proteoglycans

Recombinant viruses

Serology

SNARE/Syntaxin

Sublingual immunization

Survival

Transfection

UV radiation

Vaccines/vaccination
A7/A9 species groups
**Antibody detection**

**Antibody neutralization assay**


**Calreticulin**

**Capsid variants**


**Capsomer vaccines**

**DNA vaccines**


**E2 epitopes**

**Fusion protein**
Gardasil®

Immunity and immune responses
Peng, S., Ma, B., Chen, S-H., Hung, C-F. and Wu, T.C. (2011) DNA vaccines delivered by human papillomavirus pseudovirions as a promising approach for generating antigen-specific CD8+ T cell immunity Cell Biosci., 1: 26

Immunogenicity enhancement
L1 antibodies


L2 antibodies


LPS-derivative (non-toxic)


Measles-vectored vaccine

Memory B cells

MUC1 peptide – VLP conjugate

Placental malarial antigen

Plants, virus production in

Salmonella-based

Thermostability

Vaccination status

VLP-vaccines

Yeast-expressed

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