

OptiPrep™ Reference List RS11

Extracellular vesicles from non-mammalian sources

1. Introduction

It is widely recognized that mammalian cells, bacteria, algae and fungi release extracellular vesicles into the surrounding medium; these vesicles are involved in communication between cells and the delivery of biologically and clinically important molecules to other cells. With regard to bacteria the term “extracellular vesicles” (EVs) covers the outer membrane vesicles (OMVs) produced by Gram-negative bacteria and the membrane vesicles (MVs) produced by Gram-positive bacteria and other organisms such as algae and fungi. In all cases; EVs are distinct from the intracellular vesicles present in the cytoplasm. The OMVs from Gram-negative bacteria in particular are widely researched and have been shown to be important in the transfer of virulence factors and the initiation of immune and inflammatory responses in host cells.

- ◆ **Section 2** briefly reviews the current OptiPrep™-based methodology; this is described in detail in **Application Sheet S60**, which has its own short reference list.
- ◆ The principal aim of this Mini-Review however is to provide a bibliography of all the published papers that have reported the use of an iodixanol gradient. Published papers have been divided into the following sections:
 - ◆ **Section 3a: Gram –ve bacteria**
 - ◆ **Section 3b: Gram +ve bacteria and mycobacteria**
 - ◆ **Section 3c: Bacterial exosomes derived from human fluids and waste water**
 - ◆ **Section 3d: Haloarchaea (halobacteria)**
 - ◆ **Section 3e: Biofilm structures**
 - ◆ **Section 3f: Mycoplasmas**
 - ◆ **Section 3g: Protozoa, algae, fungi and archaea**
 - ◆ **Section 3h: Invertebrates**
 - ◆ **Section 3i: Trematodes**
 - ◆ **Section 3j: Nematodes**
 - ◆ **Section 3k: Arthropoda**
 - ◆ **Section 3l: Plants**
- ◆ **Section 4: References to methodological and functional reviews**
- ◆ **All references are listed alphabetically according to first author**

Related research areas that have reported the use of gradients prepared from OptiPrep™ are:

- ◆ The analysis of the microvesicles that are expressed from the surface of mammalian cells is covered in **OptiPrep™ Reference List RS10 and OptiPrep™ Application Sheet S63**
- ◆ The control and organization of membrane trafficking within mammalian cells that permits the movement of vesicles to, and ultimately their fusion with, the plasma membrane or a specific plasma membrane domain is covered in **OptiPrep™ Application Sheet S47**
- ◆ These and other **OptiPrep™ Reference Lists** or **OptiPrep™ Application Sheets** can be accessed via the relevant OptiPrep™ Index from the following website: www.Optiprep.com, click on “**Reference Lists**” or “**Methodology**” respectively.

2. Methodological summary

Various forms of pre-gradient processing are employed, during which intact bacteria and aggregated material in the culture medium are mostly removed and the EVs concentrated. This is covered in much greater detail in [Application Sheet S62](#)

The first step is clarification of the bacterial broth to remove intact cells by centrifugation; the time and *g*-force used varies widely and reflects the size of the organism. Generally rcf's of approx. 10,000 *g* are used for 10-20 min, but there are examples both of lower and higher *g*-forces so that the range spans 4,000-12,000 *g*. Occasionally this first step is carried out in two stages in which the broth is centrifuged for 30 min at 5,000 *g* and then the supernatant at 7,500 *g*. This strategy may minimize the entrapment (and consequent loss) of vesicles into the pellet by the rapidly sedimenting much larger bacteria. Fungal cells appear to sediment satisfactorily at lower speeds such as 2,500 *g* for 10 min.

Commonly the second step is volume reduction since ultimately the vesicles will be sedimented in a fixed-angle ultracentrifuge rotor, prior to gradient purification. Large volumes of clarified medium can pose a problem for this final pre-gradient step. The Beckman 45Ti for example which accommodates 6x94 ml tubes has a maximum *g*-force of 235,000 *g*, but the vesicles at the top of the sample experience initially only 80,000 *g*. Tangential filtration devices (e.g. 70-100 kDa cut-off) are popular or centrifugal filters are popular for this volume reduction step.

Residual bacteria in the broth may then be removed by vacuum filtration through either a 0.45 or 0.22 μm filter or both pore size filters sequentially, before the EVs are sedimented in a fixed-angle rotor; the conditions vary quite widely, from approx. 40,000 *g* for 1 h to 140-150,000 *g* for 2-3 h..

Ammonium sulphate precipitation of OMVs from the clarified broth of Gram-ve bacteria has been used a few cases. The precipitation process may only require about half an hour at 4°C but can be as long as overnight. Moreover the resuspended pellet requires dialysis overnight prior to further processing.

Iodixanol gradient purification of the EVs, regardless of the pre-gradient technology, involves adjustment of the suspension to a density of approx. 1.241 g/ml (sometimes higher 1.267 g/ml or lower 1.215 g/ml).for subsequent flotation through a discontinuous iodixanol gradient; During centrifugation at 100-200,000 *g* for 12-16 h the gradient will become more or less continuous; gradients run for shorter times (e.g. 2 h) will retain some of their discontinuous nature.

The density of MVs in iodixanol gradients is generally >1.11 g/ml, many banding between 1.13 and 1.15 g/ml; although occasionally densities of as high as 1.20 g/ml have been observed. There is also evidence of heterogeneity amongst EV populations from a single bacterial type.

The first published paper to describe the use of OptiPrep™, of which we have a record, was by Horstman and Kuehn, published in 2000. It documented the purification of OMVs from enterotoxin-containing *Escherichia coli*; the OMVs were shown to contain the pathogenic enterotoxin, lipids and specific proteins characteristic of the outer membrane and proteins from the periplasmic space, but no markers of the cytosol or inner membrane ([see Section 3a - Escherichia coli](#)).

3. Bibliography of publications reporting analytical studies on iodixanol-purified OMVs and MVs

References are sorted alphabetically according to first author. [Research topics are highlighted in blue in the titles.](#)

3a. Gram-negative bacteria

Acetobacter pasteurianus

Hashimoto, M., Matsumoto, T., Tamura-Nakano, M., Ozono, M., Hashiguchi, S. and Suda, Y. (2018) [Characterization of outer membrane vesicles of Acetobacter pasteurianus NBRC3283](#) J.Biosci. Bioeng., **125**, 425-431

Acinetobacter baumannii

- Chatterjee, S., Mondal, A., Mitra, S. and Basu, S.** (2017) *Acinetobacter baumannii* transfers the *bla_{NDM-1}* gene via outer membrane vesicles J. Antimicrob. Chemother., **72**, 2201–2207
- Marion, C.R., Lee, J., Sharma, L., Park, K-S., Lee, C., Liu, W., Liu, P., Feng, J., Gho, Y.S. and Dela Cruz, C.S.** (2019) *Toll-like receptors 2 and 4* modulate pulmonary inflammation and host factors mediated by outer membrane vesicles derived from *Acinetobacter baumannii* Infect. Immun. **87**: e00243-19
- Wachino, J-i., Jin, W., Kimura, K. and Arakawa, Y.** (2019) *Intercellular transfer of chromosomal antimicrobial resistance genes* between *Acinetobacter baumannii* strains mediated by prophages Antimicrob. Agents Chemother., **63**: e00334-19

Aggregatibacter actinomycetemcomitans

- Kieselbach, T. and Oscarsson, J.** (2017) Dataset of the proteome of purified outer membrane vesicles from the human pathogen *Aggregatibacter actinomycetemcomitans* Data in Brief, **10**, 426-431
- Rompikuntal, P.K., Thay, B., Khan, M.K., Alanko, J., Penttinen, A-M., Asikainen, S., Wai, S.N. and Oscarsson, J.** (2012) *Perinuclear localization* of internalized outer membrane vesicles carrying active cytolethal distending toxin from *Aggregatibacter actinomycetemcomitans* Infect. Immun., **80**, 31-42

Alteromonas

- Billar, S.J., McDaniel, L.D., Breitbart, M., Rogers, E., Paul, J.H. and Chisholm, S.W.** (2017) Membrane vesicles in sea water: heterogeneous DNA content and implications for viral abundance estimates ISME J., **11**, 394–404

Bacteroides vulgaris

- Maerz, J.K., Steimle, A., Lange, A., Bender, A., Fehrenbacher, B. and Frick, J-S.** (2018) Outer membrane vesicles blebbing contributes to *B. vulgatus* mpk-mediated immune response silencing Gut Microbes., **9**(1), 1–12

Borrelia burgdorferi

- Coleman, J.L., Crowley, J.T., Toledo, A.M. and Benach, G.L.** (2013) The *HtrA* protease of *Borrelia burgdorferi* degrades outer membrane protein *BmpD* and chemotaxis phosphatase *CheX* Mol. Microbiol., **88**, 619–633
- Crowley, J.T., Toledo, A.M., LaRocca, T.J., Coleman, J.L., London, E. and Benach, J.L.** (2013) Lipid exchange between *Borrelia burgdorferi* and host cells PLoS Pathog., **9**: e1003109
- Toledo, A., Coleman, J.L., Kuhlow, C.J., Crowley, J.T. and Benach, J.L.** (2012) The *enolase* of *Borrelia burgdorferi* is a plasminogen receptor released in outer membrane vesicles Infect. Immun., **80**, 359-368

Burkholderia glumae

- Kang, Y., Goo, E., Kim, J. and Hwang, I.** (2017) Critical role of quorum sensing-dependent glutamate metabolism in homeostatic osmolality and outer membrane vesiculation in *Burkholderia glumae* Sci. Rep., **7**: 44195

Burkholderia pseudomallei

- Nieves, W., Heang, J., Asakrah, S., Höner zu Bentrup, K., Roy, C.J. and Morici, L.A.** (2010) Immuno-specific responses to bacterial elongation factor *Tu* during *Burkholderia* infection and immunization PloS One **5**: e14361

Campylobacter jejuni

- Jang, K-S., Sweredoski, M.J., Graham, R.L.J., Hess, S. and Clemons Jr., W.M.** (2014) Comprehensive proteomic profiling of outer membrane vesicles from *Campylobacter jejuni* J. Proteom., **98**, 90-98

Edwardsiella tarda

- Chen, S., Yang, D., Wen, Y., Jiang, Z., Zhang, L., Jiang, J., Chen, Y., Hu, T., Wang, Q., Zhang, Y. and Liu, Q.** (2018) *Dysregulated hemolysin* liberates bacterial outer membrane vesicles for cytosolic lipopolysaccharide sensing PLoS Pathog., **14**: e1007240

Edwardsiella piscicida

- Wen, Y., Chen, S., Jiang, Z., Wang, Z., Tan, J., Hu, T., Wang, Q., Zhou, X., Zhang, Y., Liu, Q. and Yang, D.** (2019) *Dysregulated haemolysin* promotes bacterial outer membrane vesicles-induced pyroptotic-like cell death in zebrafish Cell. Microbiol., **21**: e13010

Escherichia coli

- Balsalobre, C.**, Silvan, J.M., Berglund, S., Mizunoe, Y., Uhlin, B.E. and Wai, S.N. (2006) *Release of the type I secreted α -haemolysin via outer membrane vesicles from Escherichia coli* Mol. Microbiol., **59**, 99-112
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Flavobacterium columnare

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Francisella novicida

McCaig, W.D., Koller, A. and Thanassi, D.G. (2013) *Production of outer membrane vesicles and outer membrane tubes by Francisella novicida* J. Bacteriol., **195**, 1120-1132

Francisella tularensis

Chen, L., Valentine, J.L., Huang, C-Jr., Endicott, C.E., Moeller, T.D., Rasmussen, J.A., Fletcher, J.R., Boll, J.M. et al (2016) *Outer membrane vesicles displaying engineered glycotopes elicit protective antibodies* Proc. Natl. Acad. Sci. USA, **113**, E3609–E3618

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Fusobacterium nucleatum

Liu, J., Hsieh, C-L., Gelincik, O., Devolder, B., Sei, S., Zhang, S., Lipkin, S.M. and Chang, Y.F. (2019) *Proteomic characterization of outer membrane vesicles from gut mucosa derived Fusobacterium nucleatum* J. Proteom., **195**, 125–137

Haemophilus influenzae

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Haemophilus parasuis

McCaig, W.D., Loving, C.L., Hughes, H.R. and Brockmeier, S.L. (2016) *Characterization and vaccine potential of outer membrane vesicles produced by Haemophilus parasuis* PLoS One **11**: e0149132

Helicobacter pylori

Choi, H-I., Choi, J-P., Seo, J., Kim, B.J., Rho, M., Han, J.K. and Kim, J.G. (2017) *Helicobacter pylori-derived extracellular vesicles increased in the gastric juices of gastric adenocarcinoma patients and induced inflammation mainly via specific targeting of gastric epithelial cells* Exp. Mol. Med., **49**, e330

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Klebsiella pneumoniae

Cahill, B.K., Seeley, K.W., Gutel, D. and Ellis, T.N. (2015) *Klebsiella pneumoniae* O antigen loss alters the outer membrane protein composition and the selective packaging of proteins into secreted outer membrane vesicles *Microbiol. Res.*, **180**, 1–10

Legionella pneumophila

Fernandez-Moreira, E., Helbig, J.H. and Swanson, M.S. (2006) Membrane vesicles shed by *Legionella pneumophila* inhibit fusion of phagosomes with lysosomes *Infect. Immun.*, **74**, 3285-3295

Lysobacter enzymogenes

Meers, P.R., Liu, C., Chen, R., Bartos, W. Davis, J. Dziedzic, N. Orciuolo, J., Kutyla, S., Jose, M. et al (2018) Vesicular delivery of the antifungal antibiotics of *Lysobacter enzymogenes* C3 *Appl. Environ. Microbiol.*, **84**, e01353-18

Marinobacter guineae

See “*Shewanella livingstonensis*”

Mycobacteria

See Section 3b

Neisseria gonorrhoeae

Pérez-Cruz, C., Delgado, L., López-Iglesias, C. and Mercade, E. (2015) Outer-inner membrane vesicles naturally secreted by Gram-negative pathogenic bacteria *PLoS One*, **10**: e0116896

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Neisseria meningitidis

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Porphyromonas gingivalis

Cecil, J.D., O’Brien-Simpson, N.M., Lenzo, J.C., Holden, J.A., Chen, Y-Y., Singleton, W., Gause, K.T., Yan, Y., Caruso, F. and Reynolds, E.C. (2016) Differential responses of pattern recognition receptors to outer membrane vesicles of three periodontal pathogens *PLoS One* **11**: e0151967

Prochlorococcus

Billler, S.J., McDaniel, L.D., Breitbart, M., Rogers, E., Paul, J.H. and Chisholm, S.W. (2017) Membrane vesicles in sea water: heterogeneous DNA content and implications for viral abundance estimates *ISME J.*, **11**, 394–404

Pseudoalteromonas

See “*Shewanella livingstonensis*”

Pseudomonas aeruginosa

Ballok, A.E., Filkins, L.M., Bomberger, J.M., Stanton, B.A. and O’Toole, G.A. (2014) Epoxide-mediated differential packaging of *Cif* and other virulence factors into outer membrane vesicles *J. Bacteriol.*, **196**, 3633–3642

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Acholeplasma laidlawii

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