**Advantages and limitations of using Caco-2 cells for *in vitro* M cell model**

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Oral vaccine development is reliant upon specific targeting of antigens to the gut associated lymphoid tissue (GALT). GALT is comprised of isolated and aggregated lymphoid follicles. Follicle associated epithelia (FAE) contains both enterocytes and specialized M cells, which perform the key role of luminal sampling and transport of antigens to lymphoid tissues cells beneath (FAE), initiating the mucosal immune response. The antigen-sampling M-cells are exploited by a number of pathogens including *Salmonella* (Autenrieth et al., 1996) or *Yersinia* (Jones et al., 1994). Pathogen receptors expressed by M cells have potential as target for the delivery of vaccine antigens. In this study we used the human colon adenocarcinoma cell line, Caco-2 cells, co-cultured with Raji B cells to stimulate differentiation of M cell like cells, in order to model this interface *in vitro.*

Transwell based Caco-2/Raji B cell culture model (Gullberg, 2000, Mack et al. 2009) allow for the study of M cells *in vitro* and can play a crucial role in the development of targeted oral vaccines (Tyrer, 2007). However Caco-2 cells are polyclonal nature and this high diversity has resulted in the need for standardised protocols (concerning passage number, time of usage postseeding and cell source) to be strictly followed. Caco-2 cells were also found to migrate through pores of transwell membranes as evidenced by election microscopy showing cells present on both sides of the transwell membrane. Presence of a second cell layer should be considered when using *in vitro* M cell models. Therefore, Caco-2 cells were cultured in transwells positioned in either an inverted or upright orientation to investigate the effect on Caco-2 cell migration through membrane.

Bacterial transport of *E. coli* HMN 075 (either live or killed) and killed non-typeable *Haemophilus influenzae* (NTHi) were examined using these models in order to confirm model functionality for investigating transcytosis and cell signalling pathways in M cell like cells.

References
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