DEVELOPING FLOW CYTOMETRY BASED IN SITU HYBRIDISATION (PRIMEFLOW™) TO DETERMINE EARLY INTERACTIONS BETWEEN HIV AND ITS TARGET CELLS IN HUMAN TISSUE

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Background:
The human genital and anorectal tracts make up the portals of HIV entry. These tissues contain a complex array of HIV target cells including multiple subsets of mononuclear phagocytes and CD4 T cells. However, due to difficulty of access to these human tissue types as well as technological limitations, surprisingly little is known about the very early events in HIV transmission and how HIV crosses mucosal surfaces**.** Identifying the initial HIV target cells will be essential for guiding the development of a vaccine and better PrEP regimens.

Our group has unique access to all the human tissues that HIV encounters during sexual transmission (labia, vagina, glans penis, foreskin, penile urethra, anus and rectum). Using multi-parameter flow cytometry we have characterised the specific subsets of HIV target cells that inhabit these tissues and noted tissue specific differences. Recently, we have optimised in situ hybridisation technology with microscopy (RNAscope™) in order to enable us to visualise individual HIV virions being taken up by HIV target cells as early as 30 minutes post infection.

We are now optimising this technology for use with flow cytometry (Primeflow™). This is important as unlike microscopy, it will allow us to generate quantitative data and it will also allow us to use our gating strategies to specifically identify the specific subsets of cells infected. This is not possible by microscopy which is restricted to 3 or 4 colours. Currently, we have used this assay to determine which subsets of mononuclear phagocytes take up the virus and correlated this with their ability to transfer HIV to CD4 T cells.

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