

## Abstract

Female genital tract secretions are commonly sampled by lavage of the ectocervix and vaginal vault or via a sponge inserted into the endocervix for evaluating inflammation status and immune factors critical for HIV microbicide and vaccine studies. This study uses a proteomics approach to comprehensively compare the efficacy of these methods, which sample from different compartments of the female genital tract, for the collection of immune factors. Matching sponge and lavage samples were collected from 10 healthy women and were analyzed by tandem mass spectrometry. Data was analyzed by a combination of differential protein expression analysis, hierarchical clustering and pathway analysis. Of the 385 proteins identified, endocervical sponge samples collected nearly twice as many unique proteins as cervicovaginal lavage (111 vs. 61) with 55% of proteins common to both (213). Each method/site identified 73 unique proteins that have roles in host immunity according to their gene ontology. Sponge samples enriched for specific inflammation pathways including acute phase response proteins ( $p=3.37 \times 10^{-24}$ ) and LXR/RXR immune activation pathways ( $p=8.82 \times 10^{-22}$ ) while the role IL-17A in psoriasis pathway ( $p=5.98 \times 10^{-4}$ ) and the complement system pathway ( $p=3.91 \times 10^{-3}$ ) were enriched in lavage samples. Many host defense factors were differentially enriched ( $p < 0.05$ ) between sites including known/potential antimicrobial factors ( $n=21$ ), S100 proteins ( $n=9$ ), and immune regulatory factors such as serpins ( $n=7$ ). Immunoglobulins ( $n=6$ ) were collected at comparable levels in abundance in each site although 25% of those identified were unique to sponge samples. This study demonstrates significant differences in types and quantities of immune factors and inflammation pathways collected by each sampling technique. Therefore, clinical studies that measure mucosal immune activation or factors assessing HIV transmission should utilize both collection methods to obtain the greatest representation of immune factors secreted into the female genital tract.