ABSTRACT

BACKGROUND:

Functional analysis of mononuclear leukocytes in the female genital mucosa is essential for understanding the immunologic effects of HIV vaccines and microbicides at the site of HIV exposure. However, the best female genital tract sampling technique is unclear.

METHODS AND FINDINGS:

WE ENROLLED WOMEN FROM FOUR SITES IN AFRICA AND THE US TO COMPARE THREE GENITAL LEUKOCYTE SAMPLING METHODS: cervicovaginal lavages (CVL), endocervical cytobrushes, and ectocervical biopsies. Absolute yields of mononuclear leukocyte subpopulations were determined by flow cytometric bead-based cell counting. Of the non-invasive sampling types, two combined sequential cytobrushes yielded significantly more viable mononuclear leukocytes than a CVL ($p<0.0001$). In a subsequent comparison, two cytobrushes yielded as many leukocytes (~10,000) as one biopsy, with macrophages/monocytes being more prominent in cytobrushes and T lymphocytes in biopsies. Sample yields were consistent between sites. In a subgroup analysis, we observed significant reproducibility between replicate same-day biopsies ($r=0.89$, $p=0.0123$). Visible red blood cells in cytobrushes increased leukocyte yields more than three-fold ($p=0.0078$), but did not change their subpopulation profile, indicating that these leukocytes were still largely derived from the mucosa and not peripheral blood. We also confirmed that many CD4(+) T cells in the female genital tract express the α4β7 integrin, an HIV envelope-binding mucosal homing receptor.

CONCLUSIONS:

CVL sampling recovered the lowest number of viable mononuclear leukocytes. Two cervical cytobrushes yielded comparable total numbers of viable leukocytes to one biopsy, but cytobrushes and biopsies were biased toward macrophages and T lymphocytes, respectively. Our study also established the feasibility of obtaining consistent flow cytometric analyses of isolated genital cells from four study sites in the US and Africa. These data represent an important step towards implementing mucosal cell sampling in international clinical trials of HIV prevention.