Abstract HIV-1 genital shedding is associated with increased HIV-1 transmission risk. Inflammation and ulceration are associated with increased shedding, while highly active antiretroviral therapy (HAART) has been shown to have a protective effect. We sought to examine the impact of cervical biopsies, a routine component of cervical cancer screening, on HIV-1 genital RNA levels in HIV-infected women on HAART. We enrolled HIV-1-infected women undergoing cervical biopsy for diagnosis of cervical intraepithelial neoplasia (CIN) 2/3 in this prospective cohort study. All were stable on HAART for at least 3 months. Clinical and demographic information as well as plasma HIV-1 viral load were collected at the baseline visit. Specimens for cervical HIV-1 RNA were collected immediately prior to biopsy, and 2 and 7 days afterward. Quantitative PCR determined HIV-1 concentration in cervical specimens at each time point to a lower limit of detection of 40 copies/specimen. Among the 30 participants, five (16.6%) women had detectable cervical HIV-1 RNA at baseline, of whom four (80%) had detectable HIV-1 RNA after cervical biopsy, with no significant increase in viral load in the follow-up specimens. Only one woman (3.3%) with undetectable baseline cervical HIV-1 RNA had detection postbiopsy. Detectable plasma HIV-1 RNA was the only factor associated with baseline cervical HIV-1 RNA. In women on HAART, an increase in cervical HIV-1 RNA detection or concentration was not associated with cervical biopsy. These findings help provide safety data regarding cervical cancer screening and diagnosis in HIV-infected women and inform postprocedure counseling.