Introducing Quantitative estimation of the protective effect of HSV-2 infection against reinfection with other HSV-2 strains is an important parameter for HSV-2 vaccine development. We determined the prevalence of and risk factors for HSV-2 superinfection using a novel genotyping tool.

Methods We first identified 96 high quality HSV SNPs that could determine whether HSV-2 strains were matched with >90% probability via next generation sequencing of 39 genital lesion swabs. These SNPs were then used to create a customised high throughput genotyping assay (GoldenGate, Illumina®). Two genital specimens collected from the same participant, each containing ≥5 log10 copies HSV DNA/ml, were genotyped. HIV-infected and HIV-uninfected participants participating in studies in the USA, Africa, and Peru were included. Sample pairs were excluded if <90% SNP calls were valid. Participants were considered to be infected with more than one strain of HSV-2 if their samples differed by ≥3 SNPs between the paired samples.

Results Paired genital swab specimens from 123 persons were analysed; 113 (92%) had the same strain detected at the two time points; 93 (76%) had identical SNP patterns, 18 (15%) had disagreements at one SNP, and 2 (2%) had disagreements at 2 SNPs. Ten persons (8%) were infected with more than one strain, with paired samples disagreeing at a median of 23 SNPs (range 5–33), for a minimum estimated superinfection prevalence of 8%. Of the 10 persons with HSV-2 superinfection, 7 (70%) were women and 7 (70%) were HIV infected; 6 were from Africa, one was from the USA, and 3 were from Peru.

Conclusion We developed a custom genotyping assay that provides a high throughput method for genotyping HSV-2. HSV-2 superinfection was detected in 8% of paired samples, suggesting that naturally occurring immunity to HSV-2 may not be highly efficient to protect against reinfection, especially among HIV-infected persons.

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