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009.4 ESTIMATING HSV-2 SUPERINFECTION USING A NOVEL CUSTOM GENOTYPING PLATFORM

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Introduction 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 HSV-2 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 $\geq 5 \log_{10}$ copies HSV DNA/ml, were genotyped. HIV-infected and HIV-uninfected persons 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.

Disclosure of interest statement This study was funded by the US National Institutes of Health. No pharmaceutical grants were received for the conduct of this study.

009.5 ISOLATION AND AMPLIFICATION OF TREPONEMAL DNA FOR WHOLE GENOME SEQUENCING DIRECTLY FROM THE PATIENT SAMPLE

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Introduction Syphilis, caused by *Treponema pallidum* ssp. *pallidum* (TPA), is a sexually transmitted multistage disease. Over 10 million new infections worldwide are reported every year. To date, complete genome sequences of 6 TPA strains (all passed through rabbits) have been determined. Here we present the preparation of human clinical sample for NGS without the need of TPA multiplication in rabbits.

Methods The primary chancre swab was received from Department of Dermatovenereology, St. Anne's Faculty Hospital in Brno, Czech Republic. Whole genome amplification (WGA) was carried out by multiple displacement amplification (MDA) with phi 29 polymerase after specific separation of TPA on the cell level from the human cells. Nested PCR for *polA* for detection of number of TPA DNA copies was performed.

Results MDA was not successful before separation of TPA from human cells through the inhibition of TPA amplification. Experimental addition of human DNA (3 ng) to the TPA DNA (10 ng) decreased the TPA amplification over 100 times. Therefore we apply MDA method after specific separation TPA on the cell level. Through this procedure we were able to prepare treponemal DNA (in concentration 1 ng/μl) for NGS isolated directly from the patient without the need of TPA propagation in rabbits.

Conclusion Since all yet available whole genome sequences of TPA comes from bacteria multiplied in rabbits, sequencing of syphilis genomic DNA isolated directly from the patient is required. Here we report, for the first time, the procedure for preparation of TPA DNA for NGS.

No conflicts of interest.

010 - *Trichomonas vaginalis*

010.1 TV IN PRIMARY CARE: IS THERE MORE OUT THERE THAN YOU THINK?

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Introduction The Aptima TV NAAT test has recently been approved for use (sensitivity ~100%).

Current microbiological testing involves wet mount microscopy (sensitivity 50%) or culture (sensitivity 75%). In practice, sensitivity rates may often be lower than this, due to deterioration of specimens during transport to the laboratory. Tests for *Trichomonas vaginalis* (TV) are often not performed on samples submitted from primary care because the prevalence is assumed to be too low for testing to be cost effective.

The study objective was to determine the positivity of TV in women at risk of an STI, using Aptima TV NAAT in the following groups