Methods Urine samples from men, 10 patients with urethritis and >5 PMNL/hpf and 10 healthy controls with <5 PMNL/hpf, were collected. All samples were tested for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *U parvum*, *Trichomonas vaginalis*, Herpes Simplex Virus type 1 and 2 and Adenovirus using specific PCR assays. The V3 and V4 regions of the 16S rRNA gene were PCR amplified, tagged and sequenced using the Titanium kit and GS FLX pyrose-quencing system (Roche) according to manufacturer's instructions. Sequences were analysed using the RDP Pyrosequencing Pipeline and CLC Genomics Workbench.
Results From each of the 20 samples, 8150 quality filtered sequences were randomly selected. Sequences were assigned to the genus level using the RDP Classifier. A total of 172 gen-era were identified, 133 in patients and 104 in controls. The median number of genera was 35.5 (19–49) in patients with urethritis compared to 25 (16–43) in the controls. No single genus was present in all samples. Members of the genera Pseudomonas and Sphingomonas were present in all the controls and in most of the patient samples. Also, representatives of the genera Brevundimonas, Micrococcus, Bradyrhizobium and Chryseobacterium were present in 15–18 of the samples. Of the 172 genera, 77 were only found in a single sample and 33 were found in two different samples. Rarefaction analysis at the 5 % level (comparable to genus) suggested that all the controls and most urethritis samples harboured each between 31 and 125 different groups. Four of the urethritis samples apparently contained between 150 and 300 different groups.
Conclusions Urine, even from healthy men, contains a very diverse micro-flora. Though not statistically significant, the total and median number of genera was found higher in patients with urethritis than in controls. Several widespread genera are likely to represent commensals and bacteria present in the environment.

P4-S4.02 A 22-ORGANISM MICROARRAY APPROACH FOR DETECTING MICROBIOLOGICAL ASSOCIATIONS WITH SYMPTOMATIC URETHRITIS IN MALES

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[1]S Patel, [2]M Pond. *[1]St George's Healthcare NHS Trust, London, UK; [2]St George's, University of London, London, UK*

Background In addition to its known microbiological aetiology, urethritis in men may be linked with other genital tract organisms, as yet unidentified in its pathogenesis. We used a microarray, with capacity to detect 22 genital tract organisms, in order to determine the association of symptomatic urethritis with infection or carriage of these organisms.
Methods 129 patients were asked to provide an extra first void urine specimen or give permission for their residual urine specimen submitted for *Chlamydia trachomatis* NAAT testing to be utilised. Patients were categorised into three self-reported symptom groups: definite symptoms of urethritis (discharge and/or dysuria), category 1(C1) n=80; non-specific symptoms of urethritis (eg, minimal urethral discomfort), category 2 (C2) n=26; and asymptomatic category 3 (C3) n=23. Total urine nucleic acid was extracted and subsequently used for PCR coupled microarray analysis. Organisms were defined as present or absent using an online data analysis method. In a pre-planned analysis, the following categories were compared for prevalence of organisms: C1 vs C2 and C3 combined; C1 and C2 combined vs C3 using Fisher's exact test.
Results One or more organisms were detected in 74% (n=95) of patients and two or more organisms in 33% (n=42). The prevalence of organisms known to cause urethritis in this largely symptomatic cohort was: 16% (n=21), 9% (n=12) and 5% (n=6) for *C trachomatis*, *Mycoplasma genitalium* and for *Neisseria gonorrhoeae* respectively. *Escherichia coli* was the most prevalent organism detected with a prevalence of 18% (n=23). The presence of *M genitalium* was statistically associated with C1 and C1 and C2 combined (p=0.03 and 0.01 respectively). In symptomatic patients, *C trachomatis*, *Ureaplasma urealyticum*, and *Gardnerella vaginalis* appeared to be more prevalent than in asymptomatics although not statistically significantly. Lactobacilli where detected in 1.3% and 4% of patients with C1 and C2 symptoms respectively, compared with 17% of asymptomatic patients. The absence of lactobacilli was associated with urethritis symptoms, either C1 alone or C1 and C2 combined (p=0.03 and p=0.01) respectively.
Conclusions Using a polymicrobial microarray approach we have demonstrated that symptomatic urethritis is associated with depletion of lactobacilli. This confirms early work using urethral swabs. The temporal nature of Lactobacilli depletion in relation to the onset of symptomatic urethritis needs to be investigated further.

P4-S4.03 A LOW-COST MICROFLUIDICS-BASED DIAGNOSTIC TEST FOR STDs

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S Sia. *Columbia University, New York, USA*

Background Undiagnosed and untreated STIs cause large morbidity and mortality, including birth defects and stillborn babies. Since most STI's have known treatments, the largest barriers for treating patients include high cost of transporting specimens to central labs and lack of access to diagnostic testing. We present data on a portable and low-cost microfluidics device for point-of-care diagnosis of multiple STDs (such as HIV and syphilis) in combination. In order to reduce the cost and size of the assay while maintaining high performance, we incorporated microfluidic designs such as single-use plastic microfluidic cassettes, a passive method for delivering reagents, and an amplification chemistry using gold nanoparticles.
Methods Our overall device is named mChip (mobile microfluidic chip for immunoassay on protein markers). We demonstrated an ability of mChip to simultaneously detect antibodies against HIV and syphilis in needle-pricked sample volumes. Both commercial specimens and archived specimens from Sub-Saharan Africa were used.
Results The test sensitivities and specificities for detection of HIV-specific antibodies and treponemal-specific antibodies matched the performances from lab-based ELISA. Compared to ELISA, our test can be performed anywhere, uses a very small volume of blood, and is about 10 times faster. Some of these results are in press in *Nature Medicine*, 2010.
Conclusion The mChip provided excellent performance in the diagnosis of HIV using only 1 µl of unprocessed whole-blood and <15 min assay-time, and an ability to simultaneously diagnose HIV and syphilis with sensitivities and specificities equal to lab-based assays. Overall, we demonstrate an integrated strategy for miniaturising complex laboratory assays using microfluidics and nanoparticles to enable POC diagnostics and early detection of infectious diseases in remote settings.

P4-S4.04 TEMPORAL DYNAMICS OF VAGINAL BACTERIAL COMMUNITIES

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[1]J Ravel, [1]R Brotman, [1]P Gajer, [1]J Sakamoto, [1]S Koenig, [1]L Fu, [2]X Zhou, [2]Z Abdo, [2]L Forney, [1]J Ravel. *[1]University of Maryland, School of Medicine, Baltimore, USA; [2]University of Idaho, USA*

Background Dysbiosis of vaginal bacterial communities have been associated with increased risk for sexually transmitted infections and bacterial vaginosis. This is the first observational study to model temporal dynamics of vaginal microbiota using frequently collected samples, behavioural data and culture-independent methods.