validated using 16 reference strains, representing diffent clades of the *N. gonorrhoeae* phylogenetic tree. We then studied a highly sensitive strain isolated in 1954 in Denmark in detail. Penicillin G, spectinomycin, gentamicin, tetracyline, chloramphenicol, ciprofloxacin, azithromycin, cefixime and ceftriaxone were examined in concentrations from 0.016× to 16×MIC. A pharmacodynamic function was fitted to the net bacterial growth rates at each concentration, resulting in four parameters that describe the pharmacodynamic properties of each antibiotic.

Results Our time-kill curve assay was reproducible for all the *N. gonorrhoeae* strains tested. Ciprofloxacin and spectinomycin induced the strongest bactericidal effect during the first six hours. Tetracycline and chloramphenicol were the only antibiotics that showed a purely bacteriostatic effect. Differences in the shape of the pharmacodynamic functions illustrate the time and concentration dependent properties of the antibiotics.

Conclusion We developed a standardised, robust and quality-assured time-kill curve assay for *N. gonorrhoeae*. Pharmacodynamic functions allowed classification of nine antibiotics according to their antimicrobial properties. These methods can be used to investigate new antibiotic regimens and help to improve dosing strategies.

Disclosure of interest statement This work was supported by SystemsX, Switzerland, Örebro County Council Research Committee and the Foundation for Medical Research at Örebro University Hospital, Sweden.

O09.2 ESTABLISHMENT OF THE GONORRHOEA MOUSE MODEL FOR PRE-CLINICAL TESTING OF ANTIMICROBIAL AGENTS AGAINST *NEISSERIA GONORRHOEAE*

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Introduction New antibiotics for gonorrhoea are needed due to the emergence of resistance to the extended-spectrum cephalosporins (ESCs) in *Neisseria gonorrhoeae*. Here we established the 17β-estradiol mouse model of gonococcal genital tract infection for testing antibiotics against gonorrhoea by defining the *in vivo* efficacy of cefixime (CFX) and ceftriaxone (CRO) against strain FA1090 (ESC^S) and the multi-drug resistant strain H041 (ESC^R).

Methods Estradiol-treated female BALB/c mice were inoculated vaginally with FA1090 or H041 bacteria. PBS or different doses of CFX or CRO were administered two days later (n = 9 mice/group) and vaginal swabs were quantitatively cultured for *N. gonorrhoeae* for 8 consecutive days. The percentage of mice colonised over time was compared among groups using the Log-rank test.

Results A single oral dose of 60, 12, 6 or 3 mg/kg CFX showed significant activity against strain FA1090 with the two highest doses clearing infection within 48 hr. One or two mice in the groups that received 6 or 3 mg/kg CFX did not clear infection. None of four higher concentrations (120, 60, 12, and 6 mg/kg) of CFX cleared H041 infection, but gentamycin (48 mg/kg, i.p. injection, 5 days, q24h) was effective compared to PBS. Five concentrations (30, 15, 5, 1.5, and 0.5 mg/kg) of a single i.p. dose of CRO had significant activity against FA1090, while 60, 30, 15, or 1.5 mg/kg had no effect against H041.

Conclusion The gonorrhoea mouse model shows a dose-dependent response for CRO and CFX against an ESC^S strain with *in vivo* break-points less than 0.5 and 3 mg/kg, respectively. Higher doses of these antibiotics were not effective against an ESC^R strain. We are currently correlating *in vivo* efficacy with pharmacokinetic analyses to further strengthen the usefulness of this model to test antimicrobial compounds against gonorrhoea.

Disclosure of interest statement This work was supported by NIH/NIAID (Interagency Agreement AAI14024–001) and USUHS (USU-DOD MIC73–2493).

O09.3 RELIBALE GENOTYPIC TROPISM TESTS FOR THE MAJOR HIV-1 SUBTYPES

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Introduction Human immunodeficiency virus (HIV) infects immune system cells by binding cell-surface CD4 and one of two coreceptors, CCR5 or CXCR4. Maraviroc (MVC) is an anti-HIV drug that binds to CCR5 and blocks HIV entry. Because MVC is ineffective against CXCR4-using viruses, it is only prescribed to patients shown to exclusively harbour CCR5-using viruses. The major obstacles to MVC being more widely used in anti-HIV therapies are (i) traditional pre-treatment prognostic "tropism tests" to determine CCR5- or CXCR4-usage are expensive and time consuming, and (ii) cheaper and rapid genotypic tropism tests have been developed only for subtype B viruses, which account for only 10% of infections worldwide. We developed PhenoSeq, a suite of reliable genotypic tropism tests for the major HIV subtypes; A, B, C, D and circulating recombinant forms of AE (CRF01_AE) and AG (CRF02_AG), which together account for 95% of infections worldwide.

Methods Development of genotypic tropism tests was informed by analysis of all previously published HIV genetic sequences with corresponding coreceptor usage and subtype data (n = 2257; 630 CXCR4-using and 1637 CCR5-using), to elucidate statistically significant mutations that distinguish CXCR4- from CCR5-using viruses. The accuracy of PhenoSeq was validated against independent HIV sequences from patients previously enrolled in phase III MVC clinical trials (A4001064 and MERIT), relative to phenotypic tropism tests results.

Results PhenoSeq genotypic algorithms exhibited more favourable sensitivity and specificity profiles for establishing CCR5- or CXCR4-usage of HIV subtypes A, B, C, D, CRF01_AE and CRF02_AG than alternative algorithms, including in-use algorithms geno2pheno and WebPSSM (two tailed t-test, $p \leq 0.05$ considered significant).

Conclusion As the only platform of algorithms that reliably infer tropism of all major global HIV subtypes, PhenoSeq may inform the use of MVC and future CCR5 blocking drugs, in particular for regions burdened most by the HIV pandemic, where non-B HIV predominates.

Disclosure of interest statement JFD and FD are employees of ViiV Healthcare. PRG is a former member of the ViiV Australia scientific advisory board and has received honoraria. KC and PRG have received funding from ViiV Healthcare Australia for conference travel. KC and PRG presently receive research

funding from ViiV Healthcare to further develop PhenoSeq algorithms. PRH is supported by CIHR/GSK Research Chair in Clinical Virology and has consulted and/or received grant funding from a variety pharmaceutical diagnostic companies and has received grants from, served as an ad hoc advisor to, or spoke at various events sponsored by: Pfizer, Glaxo-Smith Kline, Abbott, Merck, Selah, Tobira, Virco and Quest Diagnostics.

O09.4 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 ≥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.

O09.5 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 multiplification 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 *pol*A 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 multiplificated 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.

O10 - *Trichomonas vaginalis*

O10.1 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