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

[1,2]C Johnston*, [2,3,4,8]A Magaret, [4]C Sather, [2]K Diem, [2]M Huang, [2]S Selke, [1,5,6]JR Lingappa, [1,5,7]C Celum, [1,2,4,5]DM Koelle, [1,2,4,7]A Wald. [1]*Department of Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA, USA*; [2]*Laboratory Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA, USA*; [3]*Biostatistics, Fred Hutchinson Cancer Research Center, Seattle, WA, USA*; [4]*University of Washington, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA*; [5]*Global Health, Fred Hutchinson Cancer Research Center, Seattle, WA, USA*; [6]*Pediatrics, Fred Hutchinson Cancer Research Center, Seattle, WA, USA*; [7]*Epidemiology, Fred Hutchinson Cancer Research Center, Seattle, WA, USA*; [8]*Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA*

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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

[1]L Grillova*, [2]R Strnadel, [1]D Smajs. [1]*Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*; [2]*Department of Dermatovenereology, St. Anne's Faculty Hospital Brno, Brno, Czech Republic*

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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?

[1]J Nicholls*, [1,2]P Horner, [3]P North, [3]R Ferguson, [2]M May, [2]K Turner, [2]J Macleod, [3]P Muir. [1]*Bristol Sexual Health Centre, University Hospitals Bristol NHS Trust, Bristol, UK*; [2]*School of Social and Community Medicine, University of Bristol, Bristol, UK*; [3]*Public Health Laboratory Bristol, Public Health England, Bristol, UK*

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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

- Symptomatic genitourinary medicine (GUM)
- Asymptomatic GUM
- Symptomatic primary care
- Asymptomatic primary care

Methods The Aptima TV NAAT test was performed on 9241 samples from women undergoing chlamydia and gonorrhoea NAAT testing in GUM and primary care.
Results The positivity of TV determined by TV NAAT was 4.8% (26/543) and 1.8% (28/1593) in women with and without symptoms attending GUM and 2.7% (95/3512) and 1.1% (41/3593) respectively in primary care. TV positivity rates were high, as expected, in those of black ethnicity attending GUM (15.5% in those with symptoms). However TV positivity rates in primary care varied by practice (0–5.8%) in a way that could not be attributed to ethnicity alone.
Conclusion This is the first study to report TV positivity, using a TV NAAT, in unselected women presenting for STI testing in primary care. Positivity proportions were higher than anticipated based on conventional testing methods particularly for symptomatic women in primary care. In view of the wide variation in TV positivity by locality, other factors e.g. deprivation may be important. This should be taken into consideration should targeted testing for TV be found to be cost effective, as targeting by ethnicity alone may miss cases.
Disclosure of interest statement Hologic provided the tests for the Aptima TV NAAT research study and have sponsored the authors to present this data at ISSTDR.

O10.2 *TRICHOMONAS VAGINALIS* NUCLEIC ACID CLEARANCE FOLLOWING TREATMENT OF HIV NEGATIVE WOMEN

DH Martin*, J Burnett, SN Taylor. *LSU Health Sciences Center, New Orleans, USA*

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Background Rescreening women for *Trichomonas vaginalis* (TV) post treatment is important as repeat infections are common, ranging from 5%–31%. Nucleic acid amplification testing (NAAT) too soon after treatment may result in false positive results due to detection of remnant TV nucleic acids. The goal of this study was to determine the rate of false positive NAAT results at weeks 1–4 post treatment completion using culture as the gold standard.
Methods Women attending an STI clinic in New Orleans who were InPouch culture positive and treated with metronidazole (MTZ) were included. Participants were scheduled for 4 weekly follow up visits beginning one week post-treatment completion. They provided self-obtained vaginal swabs (SOVS) and information regarding sexual exposure at each visit. SOVS were tested using InPouch culture and the Gen-Probe AptimaTV (GPATV) assay which targets ribosomal RNA. Women who were culture positive at follow-up were considered re-infected/treatment failure and were not followed further.
Results 39 women were InPouch+ at baseline and were followed. Of these, 3 (7.7%) were InPouch TV+ at follow-up (1 at 1 week and 2 at 2 weeks) and reported no sexual exposure. Thus, these women were considered to be treatment failures and were no longer followed. Of the remaining cases, 5/29 (17.2%) were GPATV+ at the 1 week follow up visit, and 1/34 (2.9%) was GPATV+ at 2 weeks. The six positive women denied vaginal sexual re-exposure. None of the women were InPouch TV culture positive at any of the follow up visits and no woman was GPATV+ at 3 and 4 weeks post treatment.
Conclusions These data demonstrate that TV ribosomal RNA is cleared from the vagina by 3 weeks post completion of successful MTZ treatment and that the GPATV assay can be relied on as a test-of-cure at this point and beyond.
Disclosure Drs Martin and Taylor have served as consultants for Hologic Inc.

O10.3 LOW EFFECTIVENESS OF SYNDROMIC DISEASES MANAGEMENT IN WOMEN INFECTED WITH *CHLAMYDIA TRACHOMATIS*, *TRICHOMONAS VAGINALIS* AND *NEISSERIA GONORRHOEAE* LEADS IN DELHI INDIA

[1]SC Sonkar*, [1]K Wasnik, [2]A Kumar, [2]P Mittal, [1]D Saluja. *[1]Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi, India; [2]Department of Obstetrics & Gynecology Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India*

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Introduction Besides a range of effective diagnostic tests and treatments, the extent of Sexually transmitted diseases (STDs) epidemic remains challenging. STDs are associated with enormous physical, psychological and economical consequences on the population of developing countries. World Health Organization emphasises on the syndromic approach, especially in areas having inadequate laboratory and transport facilities. *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV) and *Neisseria gonorrhoeae* (NG) are the most common STIs worldwide. They present similar clinical spectra in both women and men and are the leading cause of acquired infertility in women.
Methods In this prospective study (from June 2012 to Feb 2015), the accuracy and performance of syndromic treatment given at Safdarjung hospital, as per NACO-NACP III Syndromic diagnosis of STI/RTI and treatment guidelines (Provision of directly observed therapy for single-dose regimes) were validated by comparing the diagnosis carried out by PCR based assay.
Results Out of 6000 visited patients, 820 female patients (14%) had vaginal discharge syndrome and given treatment as per NACO guidelines; using Kit-I, Grey Kit (UD, ARD, Cervivitis), Kit-II, Green Kit (Vaginitis) and Kit-VI, Yellow Kit (LAP). Out of 824, 634 (77%) patients were enrolled in this study. Based on syndromic management 20%, 0.5%, 46%, pateints were infected with CT, NG, TV respectively. Co-infections were common: 7%, 11%, 1%, 12%, with CT+TV, CT+NG, NG+TV, CT+NG+TV respectively. However, with Specific PCR assays, out of 634, 110 (17%) were positive and 524 (83%) patient were negative and/or positive for other STDs. Out of 110 patients, 7%, 5%, 2%, were CT, NG, TV infected while 1%, 2%, 1%, were co-infected with CT+TV, CT+NG, CT+NG+TV respectively.
Conclusion Our results provide evidence that, symptom based disease management leads to inaccurate diagnosis and over treatment of patients resulting in huge economic wastage and may also contribute towards the development of drug-resistance.

O10.4 PERFORMANCE OF SELF-COLLECTED PENILE SWABS FOR THE DETECTION OF *CHLAMYDIA TRACHOMATIS*, *NEISSERIA GONORRHOEAE*, *TRICHOMONAS VAGINALIS*, AND *MYCOPLASMA GENITALIUM*

[1,2]CA Gaydos*, [1]L Dize, [1]M Barnes, [1]P Barnes, [2]Y-H Hsieh, [3]D Duncan, [3]V Marsiglia. *[1]Johns Hopkins University, Infectious Diseases, Baltimore, MD, USA; [2]Johns Hopkins University, Emergency Medicine, Baltimore, MD, USA; [3]Baltimore City Health Department, Baltimore, MD, USA*

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