study was to evaluate the performance of the **cobas**® HSV 1 and 2 test in a Japanese population.

**Methods** A total of 165 specimens of anogential lesions and endocervcal swabs from 122 patients were tested from clinical sites in Tokyo, Kanagawa and Osaka (36% Female, 64% Male). Specimens were tested by **cobas**® HSV 1 and 2 test (cHSV) and compared to shell vial culture (SV) as well as Prime Check HSV Antigen Detection Kit (PCA) and direct immunofluorescence (DFA). Diagnostic agreement was evaluated and discrepant analysis was performed by Sanger sequencing.

**Results** The overall percent agreement (OPA), sensitivity and specificity of cHSV compared to SV for HSV-1 was 98.8% (95% CI 95.7–99.9), 100% (95% CI 97.8%–100%), and 98.5% (95% CI 95.3–99.8), and for HSV-2 was 87.9% (95% CI 81.9–92.5), 98.6% (95% CI 95.4%–99.8%), and 79.6% (95% CI 72.6%–85.4%), respectively. For HSV-1 there were 2 cHSV+/SV-. For HSV-2 there were 20 discordant results, 19 cHSV+/SV- and 1 cHSV-/SV+. Sanger Sequencing for HSV-1 confirmed 1 of 2 positive for HSV-1 and for HSV-2 confirmed 20/20 as HSV-2 positive. Both PCA and DFA missed more than twice as many confirmed positive HSV specimens as SV. The OPA, sensitivity and specificity of cHSV compared to DFA for HSV-1 was 84.8% (95% CI 78.4–89.9), 85.7% (95% CI 79.4%–90.7%), and 84.7% (95% CI 78.3%–89.8%), and for HSV-2 was 59.8% (95% CI 51.9%–67.4%), 100% (95% CI 97.8%–100%), and 53.2% (95% CI 45.3%–61.0%), respectively. PCA does not type positive HSV. The OPA, sensitivity and specificity of cHSV compared to PCA was 74.5% (95% CI 67.1%–81.0%), 98.8% (95% CI 95.6%–99.8%), and 51.8% (95% CI 43.9%–59.6%).

**Conclusion** This study demonstrated improved diagnostic performance of the **cobas** HSV 1 and 2 test compared to routine methods in a Japanese population.

## P1.19 HIGH-THROUGHPUT IDENTIFICATION OF SEXUALLY TRANSMITTED INFECTIONS AND BACTERIAL VAGINOSIS ASSOCIATED PATHOGENS ON OPENARRAY™ NANOFLUIDICS QPCR PLATFORM IN SOUTH AFRICA

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**Introduction** Cheap and efficient pathogen detection solutions are required to replace syndromic STI management in low and middle income countries. One solution may be point-of-care technologies at clinic level, another could be centralised high-throughput technologies. ThermoFisher recently launched the TaqMan Vaginal Microbiota Assays, in combination with OpenArray Nanofluidics qPCR platform, which is capable of testing 192 samples for 34 individual STI and bacterial vaginosis (BV) pathogens in a 2 hour qPCR run. The goal of this study was to evaluate OpenArray against an established multiplex PCR assay, and further optimise its workflow.

**Methods** Evaluation of the TaqMan Vaginal Microbiota assays on OpenArray platform was performed for 50 vaginal micobiota vaginal swab samples that had been characterised for *N. gonorrheae* (NG), *C. trachomatis* (CT), and *T. vaginalis* (TV) on an established CDC-approved multiplex PCR assay. Blind samples were provided for testing on the OpenArray platform. Nugent scores were obtained in parallel to molecular testing and results were compared for 11 specific bacterial strains indicative of BV.

**Results** High specificity (97.4%–100%) was observed at initial testing of STI samples, however the sensitivity was not as expected (NG 81.8%, CT 38.5%, TV 50.0%) due to concentrations of STI pathogens below the limit of detection on OpenArray, which was confirmed by 384-well plate testing ($C_{RT}$ range 33–38). Pre-amplification of STI samples improved the sensitivity significantly (NG 100%, CT 92%, TV 82%). Nugent scores for 46/50 samples were compared with the qPCR results for the BV-associated targets on OpenArray. BV-associated pathogens like *G. vaginalis*, *A. vaginae*, BVAB2, *Megasphaera 1*, *Megasphara 2*, *M.hominis*, and *M. mulieris* were predominate in the samples with Nugent Scores 7–10, while commensal lactobacillus were predominate in Nugent Scores 0–3.

**Conclusions** After optimisation, the OpenArray Nanofluidics qPCR platform may provide a high-throughput solution for STI pathogen detection and for characterising the vaginal microbiota.

## P1.20 ANALYSIS OF BACTERIAL DIVERSITY IN HIV/HPV COINFECTED PATIENTS WITH CERVICAL INTRAEPITHELIAL LESIONS THROUGH NEXT-GENERATION SEQUENCING

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**Introduction** Several studies have shown an increase in bacterial diversity in HPV-positive cervical cancer patients and the association of specific bacteria with cervical intraepithelial lesions. However, little is known about the cervical microbiome of HIV/HPV coinfected patients. HIV patients have a high prevalence of high-risk HPV and a greater chance of developing persistent HPV infection. The aim of this study is to evaluate the bacterial profiles of the cervical region of HIV/HPV coinfected patients, looking for a putative association of such profiles with cervical intraepithelial lesions.

**Methods** We analysed 89 $HIV^+$ cervical smear samples of women collected from 2010 to 2013. Samples have been categorised according to collection timepoint, $CD4^+$ T-cell counts and cervical intraepithelial lesions (CIN). The bacterial 16S rRNA gene was PCR-amplified and processed for next-generation sequencing in an Illumina HiSeq 2500 platform. After sequencing, reads were processed and compared against the 16S database. All bioinformatics analyses were carried out using QIIME.

**Results** The most abundant bacterial species found was *Lactobacillus iners*. We found a negative association of the *Moryella* genus with CIN, independent of the collection timepoint. On the other hand, we observed increased abundance of *Gardnerella vaginalis*, *Shuttleworthia*, *Veillonellaceae* and *Aerococcus* in CIN, but adjusted p-values were non-significant after false discovery rate and/or Bonferroni corrections.

**Conclusion** This is first study reporting the *Moryella* genus in HIV/HPV coinfected women and its potential absence in CIN.

The presence of other bacteria in CIN or in normal cervical tissues lacked significance likely due to sample size, and additional investigation is required.

## P1.21 COMPARISON OF SHIPPED VERSUS FRESHLY FROZEN SELF-COLLECTED VAGINAL SAMPLES FOR MICROBIOTA ASSESSMENT

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**Introduction** Studies have confirmed that self-collected and clinician-collected mid-vaginal swabs sample the same microbial diversity. Self-collected samples shipped through the mail for PCR-based STI testing has also been validated. We sought to determine if the microbiota of shipped vaginal samples are concordant with freshly frozen samples.
**Methods** In January-February 2016, 20 women self-collected six mid-vaginal swabs which were then stored in two different nucleic acid preservatives (3 E-swabs in 2 ml of modified C2 (MO BIO) and 3 in 2 ml of Amies/RNALater). Modified C2 was selected for ease of use in laboratory processing and DNA extraction. For each set of 3 swabs, 2 were immediately frozen (−80°C) and one was sent at room temperature to the University of Idaho in a FedEx "Clinical Pak" which was then return shipped to Baltimore. Meta-taxonomic analysis was performed by sequencing the V3-V4 regions of the 16S rRNA gene. Hierarchical clustering of vaginal microbiota was used to assign community state types (CST) to each sample. Bayesian hierarchical models were applied to perform within-women comparisons of shipped and freshly frozen samples.
**Results** Average duration of transit for the shipped samples was 8 days (SD: 1.60, range: 6–11). Paired comparison of CSTs between a woman's shipped and freshly frozen samples as well as between C2 and Amies/RNALater revealed no differences (100% concordance, kappa: 1.0 for both). After correction for multiple testing, no significant differences between phylotype relative and absolute abundances were detected in C2 or Amies/RNALater groups. Similarly, there were no statistically significant differences between total bacterial loads of shipped versus freshly frozen samples in C2 (p-value: 0.47) or Amies/RNALater (p-value: 0.21) samples.
**Conclusion** There were no differences in vaginal microbiota composition and structure between a woman's shipped and freshly frozen vaginal samples stored in Amies/RNAlater or C2. These data enable future studies to allow participants to self-collect and mail vaginal microbiota specimens.

## P1.22 VITAMIN D AND THE VAGINAL MICROBIOME: RESULTS FROM A BLINDED, RANDOMISED CONTROLLED TRIAL

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**Introduction** A blinded, randomised controlled trial (RCT) previously demonstrated no effect of vitamin D supplementation on recurrence of bacterial vaginosis (BV) diagnosed by Nugent score. Here, we examine archived samples to determine whether vitamin D supplementation affected trial participants' vaginal microbial composition.
**Methods** Women with symptomatic BV via Amsel criteria were recruited and treated with 500 mg of oral metronidazole at baseline. Participants were randomised to vitamin D supplementation (9 doses of 50,000 IU cholecalciferol over 24 weeks) or matching placebo. Vaginal bacterial composition was characterised for 15 women in each treatment arm, using samples collected at baseline, 4 and 24 weeks. We sequenced the V1-V3 region of the 16S rRNA gene and taxonomy was assigned by PECAN. Microbiota were clustered into 5 community state types (CSTs) using Bray-Curtis distances and hierarchical clustering with Ward linkage. We assessed serum vitamin D levels using the Liaison 25 OH vitamin D total assay.
**Results** We observed no significant effect of vitamin D treatment on *Lactobacillus* dominance over 24 weeks ($p>0.5$). Additionally, serum vitamin D levels were not associated with CST ($p=0.22$). Following metronidazole treatment, the *Lactobacillus iners*-dominated CST (III) was more common at week 4 compared to enrollment in both the placebo and vitamin D groups. Specifically, the relative abundance of *L. iners* was significantly higher in the placebo arm at weeks 4 ($p<0.001$) and 24 ($p=0.04$) compared to the vitamin D arm.
**Conclusion** In agreement with the RCT, we observed no association between vitamin D supplementation and the vaginal microbiota. While many women in both RCT groups tended toward *L. iners*-dominated microbiotas following metronidazole treatment at week 4, *L. iners* was significantly more abundant in the placebo group. Future research may examine if vitamin D plays a role in stimulating non-lactobacilli growth and how supplements, in addition to antibiotics, affect the emergence of robust lactic acid producing lactobacilli.

## P1.23 ANTIBIOTIC USAGE AND COMMENSAL PHARYNGEAL NEISSERIA OF MSM IN HANOI, VIETNAM

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**Introduction:** *Neisseria gonorrhoea* (NG) is gaining resistance to last line cephalosporins; conferring resistance to almost all antimicrobials used to treat it since the 1930s. NG disproportionately affects men-who-have-sex-with-men (MSM) and sex workers. Neisseria are particularly apt at horizontal gene transmission within the genus. Genetic analysis of resistant NG found fragments from *N. cinerea* and *N. perflava*, common commensals of the oropharynx. Nearly all global cases of ceftriaxone resistant NG are reported from pharyngeal samples. Self-medication with antibiotics is prevalent in Vietnam and MSM of Hanoi have high rates of STIs.
**Methods** MSM from Hanoi, Vietnam were surveyed regarding health seeking behaviours, including antibiotic usage.