**Research benefits of storing genitourinary samples: 16SrRNA sequencing to evaluate vaginal bacterial communities**

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Using well characterised, but old and carefully frozen genital tract research samples may be a cost effective way of performing metagenomic studies, but risks loss of low abundance (but relevant) bacterial species DNA. Moi and colleagues used 16SrRNA and UreDNA sequencing to detect ureaplasmas in frozen urine samples collected from 362 men with NGU in 2010-111. They found that urethral inflammatory responses to ureaplasmas were less severe than to *Chlamydia trachomatis* and *Mycoplasma genitalium*.

In a pilot study in 2015 we used 16SrRNA sequencing to evaluate bacterial communities in 20 stored self-taken vaginal samples collected in 2004-8 for the Prevention of Pelvic Infection (POPI) chlamydia screening trial.2 Mean age of women whose samples were tested was 18 years (range 16 to 26), and three were positive for *C. trachomatis,* (including one co-infected with *N. gonorrhoeae)* and one for *M. genitalium*. Despite up to 11 years storage in Gen-Probe Aptima transport medium at -80◦C, DNA integrity was adequate in all 20 samples analysed including four postal samples.

The full-length bacterial 16S rRNA gene was amplified using New England Biolab Q5 high-fidelity polymerase kit. Equimolar library mix was prepared and sequenced using PacBio SMRT sequencing technology. Downstream bioinformatics analysis was performed using Mothur (version 1.34.4).

We obtained 73,590 raw sequences from 22 samples, including one negative and one positive control, of which 41,166 were high quality. Four samples with low sequence number (<500 sequences) were excluded. In the remaining 16 samples, 65 unique oligotype species were identified, of which 14 had a minimum abundance of 0.1% (Figure 1.) Burkholderia species were identified as contaminants and removed.

Five oligotype species had a minimum abundance of 1%: *Lactobacillus crispatus* (52.8%), *Lactobacillus iners* (33.7%), *Gardnerella vaginalis* (3.5%), *Lactobacillus gasseri* (2.9%), and *Finegoldia magna* (1.1%). Six samples were dominated by *Lactobacillus iners* (red bar), and seven samples by *Lactobacillus crispatus* (blue bar). Sample 581\_lbc2, previously assessed as bacterial vaginosis (BV) intermediate, was dominated by *Gardnerella vaginalis*. Patient 1117, the one positive for *C. trachomatis* and *N. gonorrhoeae* had the highest number of *Megasphaera elsdenii*, which has been reported in BV. Patient 586\_Ibc6 described her ethnicity as black other and had the largest bacterial diversity among all patients analyzed.

We note that *C. trachomatis* and *N. gonorrhoeae* were not detected in the samples. This was not unexpected given that we sequenced from vulvo-vaginal swabs, and the source of whole organism for these pathogens in the female reproductive tract is the cervix. Although nucleic acid amplification tests using specific primers for *C. trachomatis* and *N. gonorrhoeae* is highly accurate in vulvo-vaginal swabs, the relative low abundance of their genomic DNA in these samples would mean generic 16SrRNA primers were likely to preferentially bind to higher load organsims.

Like Moi and colleagues, we found that carefully characterised samples can be used after many years storage for investigating research questions using 16SrRNA sequencing or PCR.3-5

**Ethical review**: Bromley research ethics committee reference: 07/Q0705/16

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**Figure 1.Species identified from 16 self-taken vaginal swabs after up to 11 years storage**