

with different disease phenotypes along with distinct tissue tropisms. The extra chromosomal plasmid of *Chlamydia trachomatis* is suggested to encode genes essential for chlamydial infection and transmission. Using an *in silico* plasmid MLST scheme, the clustering of *C. trachomatis* isolates was investigated in association with previously defined *ompA* biovars.

**Methods:** *In silico* analysis using publicly available whole genome sequence data (WGS) of *C. trachomatis* isolates deposited in the *Chlamydiales* pubMLST database (<http://pubmlst.org/chlamydiales/>) was performed. Only data from WGS were investigated ensuring that complete sequence data in all eight known plasmid genes and *ompA*, were included. An *in silico* plasmid MLST scheme was developed to assign allele numbers and plasmid sequence types to all included isolates. Clustering of *C. trachomatis* plasmids was assessed using minimum spanning tree analysis. Moreover, we performed a polymorphism analysis of each plasmid gene.

**Results** Using the *in silico* plasmid MLST scheme, plasmid alleles and sequence types were successfully assigned to 157 *C. trachomatis* isolates. Overall, 47 unique plasmid sequence types were detected. Minimum spanning tree analysis identified 5 large clusters, which showed clustering of *C. trachomatis* plasmids according to the *ompA* defined biovars. Further analysis of individual plasmid genes showed that besides specific STs, each biovar also had distinct plasmid alleles. Moreover, analysis of the polymorphic variation of plasmid genes confirmed that the *C. trachomatis* plasmid was highly conserved with all isolates sharing >99% sequence identity.

**Conclusion:** *In silico* multilocus sequence typing of *C. trachomatis* plasmids showed clustering of isolates according to biovars, suggesting that the *C. trachomatis* plasmid along with specific plasmid genes may play a role in the distinct disease phenotypes found in *C. trachomatis* infections.

P1.07

#### CONTRIBUTION OF PCR IN THE BIOLOGICAL DIAGNOSIS OF *TRICHOMONAS VAGINALIS* INFECTION AT INSTITUT PASTEUR OF COTE D'IVOIRE

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**Introduction** The urogenital trichomoniasis remains the most common non-viral sexually transmitted infection. Classically, its biological diagnosis relies on the use of microscopy which is often negative because of the fragility of the parasite. PCR is thus an alternative method. So, this study aims to show the importance of PCR in the diagnosis of *Trichomonas vaginalis*.

**Methods** This cross-sectional study was conducted among 194 women received at the Institut Pasteur of Côte d'Ivoire from July to October 2013 for an STD through a vaginal sample with three swabs. Direct examination was made on each sample, Gram stain and Giemsa, culture as well as a multiplex PCR (*T. vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium*). The sociodemographic and clinical data were collected using a questionnaire.

**Results** In total, 194 women were received with an average age of 31.35 years (SD=8.60 years). At microscopy, 2 cases (1.03%) of *T. vaginalis* were revealed. After DNA extraction, a PCR amplification has allowed to identify 7 cases (3, 61%)

of *T. vaginalis* with a sensitivity of 100% and specificity of 97.4%. Other germs were identified by PCR, *Chlamydia trachomatis* (4.12%), *Neisseria gonorrhoeae* (2.58%) and *Mycoplasma genitalium* (1.03%).

**Conclusion** Routine testing of *Trichomonas vaginalis* by PCR has shown the importance of this method in the diagnosis of *Trichomonas vaginalis* infection because of its high sensibility and specificity. Its might be an alternative after the initiation of classical microscopy.

P1.08

#### DEVELOPMENT, VALIDATION AND TESTING COSTS OF AN IN-HOUSE REAL-TIME POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF *CHLAMYDIA TRACHOMATIS*

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**Introduction** In Brazil, the Digene Hybrid Capture II DNA test (HCII CT-ID - Qiagen) is the only molecular test used to identify *Chlamydia trachomatis* (CT). This test has shown sensitivity and specificity ranging from 93.8%–97.7% and 95.9%–100.0%, respectively, compared to that of culture. However, up to 27.7% of negative results are false. Real time polymerase chain reaction (qPCR) offers both high sensitivity and specificity. The objective of this study is to describe the development, assess the performance, and costs, of a new in-house qPCR assay for the diagnosis of genital chlamydial infection.

**Methods** Asymptomatic women aged 14–25 years who attended primary health services in Manaus, Brazil, were screened for CT using the HCII CT-ID-test. A subset of cervical specimens were tested using an in-house qPCR and a commercial qPCR (Artus qPCR) as a reference test. A primer/probe based on the sequence of cryptic plasmid was designed. An economic evaluation was conducted from the provider's perspective. Overall, 292 specimens were tested by both the commercial kit qPCR and the in-house qPCR.

**Results** The primers and probe designed for the CP target showed specificity, because no cross-reaction was observed for other STI species tested or with the human DNA. We were able to standardise this reaction in 10 µL and 5 µL volumes for the CP and β-actin set primers, respectively. The sensitivity, specificity, positive and negative predictive values of in the in-house qPCR were 99.5% (95% confidence interval [CI]: 97.1–100), 95.1% (95% CI: 89–98.4), 97.4% (95% CI: 94–99.1), and 99.0% (95% CI: 94.5–100), respectively. The cost per case of CT was \$0.55 for HCII CT-ID, \$1.45 for Artus qPCR and \$1.33 for in-house qPCR.

**Conclusion** In conclusion, we have developed an in-house qPCR to detect cervical CT targeting CP primers. The in-house qPCR method was its lower cost. Findings from this study can help to revise the national recommendation of using HCII CT-ID. The in-house qPCR should be considered as a good candidate for the diagnostic method for screening programs in Brazil.