(BV) is a polymicrobial condition with low *Lactobacillus spp.* count and an increase in organisms such as *Mycoplasma hominis* and *Garderella vaginalis*. BV is associated with increased risk of acquisition of STDs/HIV and pregnancy-related morbidities. In this study, we described the general vaginal flora as well as the Lactobacillus spp of 10 healthy women and 10 women with BV in the Bay Area, California, USA.

Methods Between July 2009 and April 2010, we obtained vaginal swabs from 10 healthy women and 10 women with BV at the San Francisco City Clinic with informed consent. BV status was determined by Nugent scoring. The swabs were cultured in anaerobic conditions on Columbia agar, for unspecific bacterial growth, and on Rogosa agar, selective for *Lactobacillus spp*. By sequencing PCR amplifications of the 16srRNA gene, 5 to 10 single bacterial colonies from both agars were identified at the species level in all samples.

Results A total of 277 bacterial colonies were successfully sequenced from healthy women (143) and women with BV (134). A wide range of organisms were identified in both groups. Corynebacterium spp were found in healthy women (11, 7.7%) and women with BV (25, 18.5%). Enterococcus faecalis was present in both groups (BV−: 15, 10.5%; BV+: 10, 7.5%). *Streptococcus spp* were found as well (BV−: 9, 6.3%; BV+: 15, 11.6%). *Staphylococcus spp.* were isolated (BV−: 28, 19.6%; BV+: 22, 16.2%), with *S epidermis* being the most common (BV−: 15, 10.5%; BV+: 7, 5.2%). Interestingly, *Gardnerella vaginalis* was isolated in one healthy woman in addition to women with BV (7, 5.2%). *Lactobacillus spp.* were found with higher frequency in healthy women (BV−: 68, 46.2%; BV+: 28, 20.8%). The most common species in healthy women were: *L crispatus* (42, 62% of total *Lactobacillus spp.*) and *L jensenii* (11, 16.2%). In women with BV, the most common was *L coleohominis* (17, 61%) see Abstract P3-S7.19 table 1.

Conclusions This pilot study with a sample size of 20 women gave important information regarding the diversity of the vaginal flora in healthy women and women with BV. There is a clear switch in *Lactobacillus spp.* dominance in health women vs women with BV. This finding sheds light on the association of specific *Lactobacillus spp.* with bacterial vaginosis.

Abstract P3-S7.19 Table 1 Species Table US Data

Genus	Species	No. (%) of isolates in US collection BV−	BV+
Actinomyces	*neuii*	0	1 (0.7)
Brevibacterium	*casei*	0	1 (0.7)
	ravenspurgense	1 (0.7)	0
Chryseobacterium	ND	0	1 (0.7)
Citrobacter	*koseri*	3 (2.1)	1 (0.7)
	ND	1 (0.7)	0
Corynebacterium	*amycolatum*	6 (4.2)	8 (6)
	aurimucosum	1 (0.7)	8 (6)
	coyleae	2 (1.4)	3 (2.2)
	simulans	0	1 (0.7)
	tuscaniae	0	2 (1.4)
	ND	2 (1.4)	3 (2.2)
Delftia	ND	0	1 (0.7)
Enterococcus	*faecalis*	15 (10.5)	10 (7.5)
Erwinia	ND	0	1 (0.7)
Escherichia	*coli*	0	5 (3.7)
Facklamia	*hominis*	0	1 (0.7)
Gardnerella	*vaginalis*	1 (0.7)	7 (5.2)
	ND	1 (0.7)	0
Gordonia	*polyisoprenivorans*	0	1 (0.7)
Lactobacillus	*coleohominis*	1 (0.7)	17 (12.7)
	crispatus	42 (29.4)	1 (0.7)
	gasseri	0	2 (1.5)
	iners	2 (1.4)	0
	jensenii	11 (7.7)	3 (2.2)
	johnsonii	4 (2.8)	3 (2.2)
	rhamnosus	3 (2.1)	0
	ruminis	0	2 (1.5)
	vaginalis	5 (3.5)	0
Microbacterium	ND	0	2 (1.5)
Proteus	*mirabilis*	1 (0.7)	0
Staphylococcus	*aureus*	0	1 (0.7)
	condimenti	1 (0.7)	0
	epidermidis	15 (10.5)	7 (5.2)
	haemolyticus	4 (2.8)	5 (3.7)
	hominis	4 (2.8)	1 (0.7)
	lugdunensis	2 (1.4)	2 (1.5)
	pasteuri	1 (0.7)	0
	simulans	0	1 (0.7)
	ND	1 (0.7)	5 (3.7)
Streptococcus	*agalactiae*	3 (2.1)	1 (0.7)
	anginosus	3 (2.1)	9 (6.7)
	cristatus	1 (0.7)	0
	mitis	2 (1.4)	1 (0.7)
	oralis	0	1 (0.7)
	pasteurianus	0	1 (0.7)
	salivarius	0	1 (0.7)
	ND	0	1 (0.7)
Tsukamurella	*tyrosinosolvens*	0	1 (0.7)
Total		143 (100)	134 (100)

Basic sciences poster session 1: *Chlamydia trachomatis* and *Neisseria gonorrehea*

P4-S1.01 ROLE OF *CHLAMYDIA TRACHOMATIS* HEAT SHOCK PROTEINS 60 AND 10 IN INDUCTION OF APOPTOSIS IN ENDOCERVICAL EPITHELIAL CELLS

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Background Chlamydiae are known to modulate host cell to escape from immune response and prolong their persistence to cause fallopian tube damage, ectopic pregnancy and infertility. In addition, Chlamydiae have been reported to elicit both the induction of host cell death, or apoptosis, under some circumstances and actively inhibit apoptosis under others. Chlamydial heat shock proteins (cHSPs) have been known to be responsible for proinflammatory pathologic manifestations of human chlamydial disease in the reproductive tract. Moreover, cHSP60 has been shown to induce apoptosis, in vitro, in primary human trophoblasts, placental fibroblasts, and the JEG3 trophoblast cell line. However, no study has been dedicated to their potential role in apoptosis of primary cervical epithelial cells that are privilege target for chlamydial infection. In the present study, we investigated the ability of cHSP60 and cHSP10 to induce apoptosis in primary cervical epithelial cells.

Methods Primary cervical epithelial cells were stimulated with cHSP60 and cHSP10 for 4 h. Quantitative measurements of apoptosis have been performed by cytofluorometry and apoptosis-related genes were analysed by microarray, real-time PCR and western blotting. Further, levels ofd proinflammatory cytokines (IL-18 and IL-1ß) were determined by semi-quantitative RT-PCR.

Results Treatment with cHSP60 significantly increased the mean percentage of apoptotic cells (57.4±5.9 % vs 9.3±1.2 % in control cells, $p < 0.05$). Similarly, treatment with cHSP10 significantly

increased the mean percentage of apoptotic cells (47.8±4.8 % vs 9.3±1.2 % in control cells, p <0.05). A cDNA microarray study showed significant (p <0.05) upregulation of interleukin (IL)-1 ß-convertase, caspase-3, -8 and -9 genes were confirmed in real-time RT-PCR in cHSP60 and cHSP10 stimulated than in control cells. The transcript levels of IL-1ß and IL-18 in cells treated with cHSP60 and cHSP10 was found to be significantly (p <0.05) higher in stimulated than in control cells.

Conclusions In women with persistent chlamydial infection, the release of extracellular cHSPs may lead to cell apoptosis and to an inflammatory response involved in fibrosis and scarring of female genital tract that may contribute severe tubal pathologies including infertility.

P4-S1.02 COUPLING OF ELECTROCHEMICAL DETECTION WITH PCR AMPLIFICATION FOR SENSITIVE DETECTION OF *NEISSERIA GONORRHOEAE*

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Background Despite the recent development of different detection methods, better diagnostic tools are required for quick and reliable detection of pathogens. Use of biosensors for detection of pathogens is gradually gaining momentum. However, PCR amplification of DNA target is still necessary for application on biosensor for accurate detection of the pathogen. An in-house PCR using self-designed primers targeting the opa gene (GenBank accession no. PUID 9716120 SNUM 2706 Ng_opa) was performed. The generated amplicons were used to evaluate the DNA biosensor utilising a 19-mer oligonucleotide sequence (GenBank PUID SNUM: 9716119 2705 Ng_opa) as probe.

Methods In-house PCR was standardised and an amplified product (amplicons) of 188 bps were obtained for positive samples. Fabrication of bioelectrode was performed by immobilising the activated probe onto pre-treated screen-printed gold electrodes. The fabricated nucleic acid functionalised gold electrode was characterised using, SEM, CV, DPV techniques. The presence of target DNA was detected electrochemically by monitoring the redox peak of methylene blue indicator. Standardisation of working conditions was done using complementary, non-complementary, one base mismatch DNA, and amplicons from standard strain of N. gonorrhoeae (ATCC 49226) & 16 clinical isolates. In addition, DPV measurements of hybridised bioelectrode with amplicons of 26 clinical samples of which 10 were culture positive, was done. A cut-off value for positives was determined by using the software STATA (version 9).

Results The analytical sensitivity of PCR was 10−17 M of DNA and the bioelectrode could detect up to 1.0×10−20 M of the DNA amplicons. An 11.49% decrease in signal intensity was taken as the cut-off. Samples giving an equivalent or more decrease than this value were considered as positives.

Conclusions The coupling of electrochemical detection with PCR amplification showed the advantage of higher sensitivity and increased specificity for detection of *N gonorrhoeae*. This may prove to be particularly valuable for the identification of asymptomatic infections and could greatly improve gonorrhoea control.

P4-S1.03 DEMONSTRATING PERFORMANCE OF A LOW-COST, ULTRA-RAPID PCR DEVICE WITH TRUE POINT-OF-CARE APPLICATIONS

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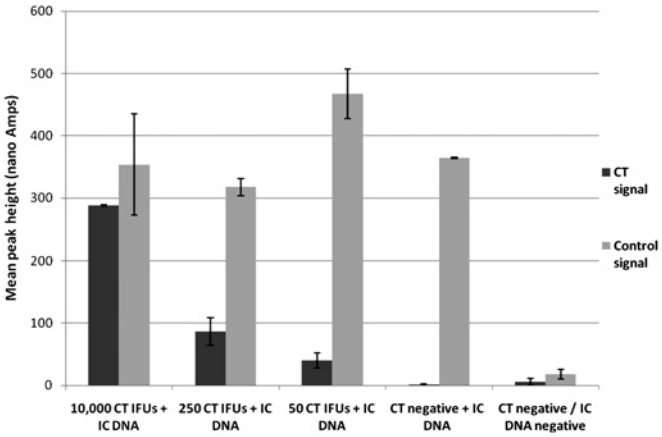
Background We are developing a highly sensitive, ultra-rapid, multiplex PCR method with fully-integrated DNA preparation and ambient-stable reagents. The assay was used in conjunction with a novel electrochemical detection method to demonstrate low copy number amplification and detection in <20 min, as a point-of-care (POC) diagnostic test for *Chlamydia trachomatis* (CT).

Methods The method employs custom PCR cards, utilising a thin-film laminate construction to achieve rapid heat transfer, in conjunction with an ultra-rapid thermocycler. All reagents necessary to perform the extraction, amplification and detection are deposited into the cards and air dried at the point of manufacture. Novel, ambient-stable reagent formulations with an 18 month shelf life have been developed. A sample is added to the card and DNA extracted from the sample. The resulting eluate reconstitutes dried PCR reagents and a 40-cycle multiplex PCR is performed using rapid thermocycling. Amplified target is detected using electrochemically-labelled target-specific probes and a double-stranded DNA-specific exonuclease to release the electrochemical label. Released label is read by applying a voltage to a screen printed carbon electrode and at a known oxidation potential the label is oxidised producing a measurable current. The unique rapid performance of this device has been demonstrated in terms of analytical sensitivity and reagent stability under ambient storage conditions. Multiplex capability is demonstrated in this test with the presence of internal control (IC) DNA.

Results Analytical sensitivity of the device was evaluated by testing dilutions of CT in the presence of IC DNA. The results show CT detection down to 50 copies when co-extracted, amplified in duplex and detected electrochemically with the IC DNA (see graph). Tests on the reagents dried into the device showed stability for 18 months when stored at ambient temperature (20–25°C). Reagent performance after 18 months' storage was shown to be equivalent to performance at time zero see Abstract P4-S1.03 figure 1.

Conclusions The results show that this device could be used to perform ultra-rapid multiplex PCR with no user intervention after sample addition, allowing minimally-trained staff to carry out the assay in <20 min, meeting the needs for a "true" POC device. Ambient stability of the reagents negates the requirement for any specialised storage conditions.



Abstract P4-S1.03 Figure 1