Systems Biology and Novel Technologies For Molecular Analysis and Diagnosis

P1.01 EVALUATION OF A NEW HOME-BASED SELF-VAGINAL COLLECTION DEVICE FOR DETECTION OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE*

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Introduction Self vaginal sampling is a new collection approach for detection of Sexually Transmitted Infections and is able to guarantee privacy and comfort during the collection. The aim of the study was to evaluate usability, vaginal cells collection efficiency and ability to preserve nucleic acids stability of a new self vaginal flocked swab (FLOQSwab™, Copan) developed for home collection.

Methods 80 donors (age 18 to 45) performed a double self-vaginal sampling (n=160) using: a certified flocked self-vaginal point of care collection (POC) device as a reference method (Copan); a new home-based self-vaginal flocked swab by following the kit instructions. Patients received a questionnaire to assess the usability of the new device. Home-based and POC self-vaginal swabs have been processed using Xpert CT/NG assay (Cepheid). The threshold cycle value (Ct) of a human genomic target (sample adequacy control), Ct of pathogens (*Chlamydia trachomatis* CT and *Neisseria gonorrhoeae* GC2 and GC4) and extraction and amplification control (*Bacillus globigii* spores) were considered to compare performance between the two devices. To evaluate the stability of the nucleic acids at time 0 and after 4 weeks of storage at +4 °C and +30 °C, 54 negative home collected self vaginal flocked samples have been inoculated with a suspension of CT and GC ATCC (VR880-43069) at 1 and 10xLOD of molecular assay.

Results 100% of overall agreement was obtained comparing the results between the two devices: 77/80 negative and 3/80 *Chlamydia trachomatis* positive patients were detected. No failure results have been observed. The survey reported a better appreciated home-based self vaginal collection (80%) with respect to the POC sampling. After 4 weeks of storage at 4°C and at 30°C all 54 spiked samples have been correctly detected.

Conclusion the new home-based self vaginal device has shown the same performance of the reference swab, demonstrating an efficient recovery of vaginal cells, stability of CT and GC nucleic acids up to 4 weeks at 4°C and +30°C and excellent acceptability by women.

P1.02 PRODUCTION OF POLYCLONALS ANTIBODIES AGAINST *GARDNERELLA VAGINALIS*

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Introduction Bacterial Vaginosis (BV) is the most frequent vaginal infection. It is characterised by a decrease in the number of Lactobacilli and an increse of anaerobic bacteria. *Gardnerella vaginalis* is the main etiological agent, this bacteria has multiple virulence factors such as the production of biofilm, sialidase and vaginolysin, which can cause the degradation of cervical mucus, adhesion and lysis of epithelial cells. The production of antibodies against this microorganism will allow understanding their role in the development of BV.

Methods Three New Zealand rabbits were immunised for 8 weeks using as antigen the strain ATCC 14018 of G. vaginalis and the complete and incomplete Freund's adjuvants. The immune response was evaluated at weeks 0, 4th and 7th by indirect ELISA. At the 8th week the rabbits were sacrificed and blood serum was obtained, purification was performed using the Protein A antibody purification kit (Sigma). For the characterisation of the policlonal antibody we perform Indirect ELISA, Dot Blot, Western Blot and inhibition of haemagglutination.

Results Two polyclonal antibodies agains *G. vaginalis* were obtained. The first was obtained from Rabbit 1 (A.ka. Gv1) and the second one is a Pool (Gv2) from the serum of rabbits 2 and 3. Both antibodies recognise the strain ATCC 14018 of *G. vaginalis* at titers greater than 1: 2000 and proteins with molecular weights of approximately 38, 50, 65, 75 and 90 kDa, in addition the antibodies are capable of inhibiting lysis of vaginolysin.

Conclusion The produced antibodies will be use to study the pathogenesis of *Gardnerella vaginalis* during the development of BV.

P1.03 CHARACTERISATION OF IMMUNOGLOBULIN A/G RESPONSES DURING 3 DOSES OF THE HUMAN PAPILLOMAVIRUS-16/18 AS04-ADJUVANTED VACCINE

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Introduction Individuals receiving the human papillomavirus (HPV) vaccine develop high levels of circulating neutralising antibodies. However, data about antibody responses in the cervix are limited.

Methods This study was designed to describe the course of IgA/IgG responses in cervical secretions and in serum after intramuscular administration of the HPV16/18 AS04-adjuvant vaccine. An enzyme linked immunosorbent assay for detection of IgA and IgG anti–HPVVLP was developed for this purpose.

Results Immunoglobulin G seroconversion after the second dose was observed in 100% of the participants and remained 1 month after the third dose. Regarding IgG reactivity in cervical secretions, conversion was observed in 85% of women after the final dose. Immunoglobulin A seroconversion was observed in 76.7% of women after the third dose. Lower levels of IgA were detected in the cervical mucus (28.3%) and decreased to 23.3% after the last dose. Comparing local and systemic IgG responses, positivity in both serum and cervical samples was observed in 85%, whereas in 15% only, the serum was IgG antibody positive. A weak agreement between local and systemic IgA responses was observed. Only 18.3% of participants were local and systemic IgA positive, 58.4% were positive only in serum, 5% were positive only in the

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cervix, and 18.3% were both local and systemic IgA antibody negative.

Conclusion After the third vaccination, there is a strong agreement between cervical and systemic IgG antibody responses and a weak agreement between cervical and systemic IgA antibody responses. The induction of IgA antibodies seems to be secondary to that of IgG antibodies in response to HPV intramuscular vaccination.

P1.04 DEVELOPMENT OF AN ELISA-ASSAY FOR DETECTION OF IGA AND IGG AGAINST HUMAN PAPILLOMA VIRUS

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Introduction The interest in human papilloma virus (HPV) seropositivity has increased considerably since HPV vaccines have become available worldwide. The aim of this study was to assess the performance of enzyme-linked immunosorbent assay (ELISA) in analysing serum samples provided from women with and without genital DNA-HPV infection confirmed by polymerase chain reaction (PCR), for detection of specific antibodies of the isotypes IgG and IgA recognising HPV-16 and –18, as well as virus-like particles (VLPs).

Methods 50 sexually active female patients between 18 and 35 years of age from the outpatient clinic at the university hospital were enrolled. In order to test them, positive controls were obtained from patients with HPV-induced lesions and who were DNA-HPV positive confirmed by PCR. A specific assay was used to identify antibodies to HPV VLPs by ELISA. The samples were divided into HPV positive and negative, and an ELISA detecting IgA and IgG anti-HPV-VLP was carried out.

Results The effectiveness of ELISA and the kappa (k) index was obtained from the values entered in the receiver operating characteristic (ROC) curves for IgG and IgA. IgG-VLP-HPV-16 showed a good correlation between ELISA and PCR (k=0.75), and IgG-VLP-HPV-18 showed a very good correlation between ELISA and PCR (k=0.84). While the IgA antibody correlation was also positive, although weaker, IgA-VLP-HPV-16 was moderate (k=0.45) and IgA-VLP-HPV-18 good (k=0.66). The efficacy of the assay concerning IgG was: sensitivity, specificity, and accuracy were 82.3%, 92%, and 88% to IgG-VLP-HPV-16, and 100%, 92%, and 94% to IgG-VLP-HPV-18. The assay concerning IgA was: sensitivity, specificity, and accuracy were 64.7%, 80%, and 73.8% to IgA-VLP-HPV-16, and 100%, 80%, and 84.8% to IgA-VLP-HPV-18.

Conclusion IgG and IgA antibodies against HPV-16 and –18 can be detected in unvaccinated individuals by using the VLP that serve as the basis for bivalent HPV vaccine. The values for ELISA assays and the values found for IgG correlate good/very good with HPV-16/18 detected by PCR.

P1.05 CURRENT USE AND PERCEIVED OBSTACLES TO USE OF POINT-OF-CARE TESTS IN SUB-SAHARAN AFRICA

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Introduction Sexually transmitted infections (STIs) continue to be a significant global public health issue. Rapid, inexpensive and easy-to-use point of care tests (POCTs) for syphilis, HIV, and trichomonas have become available and WHO pre-qualified. We surveyed public health workers from Africa to determine current STI POCT practices.

Methods From March to October 2016, we invited 7584 healthcare workers (HCW) to participate in an email survey using Qualtrics software. Email addresses were obtained from the Ministry of Health and a database of training attendees at the Infectious Diseases Institute over the last 6 years.

Results 555 participants (50% male) took the online survey. 91% were from Uganda. Most (n=449, 81%) were clinicians including 336 (61%) nurses and 113 (20%) clinical officers of Level III/IV health centres (40%). Among 449 clinician respondents, 312 (69%) reported seeing HIV/general patients and 309 (70%) diagnose at least one patient with an STI daily. Of 555 participants, common POCTs used were pregnancy test (74%), urine dipstick (71%), syphilis rapid test (66%) and Gram's stain (53%). Clinicians reported that 86% of POCTs were performed by HCWs and 54% read the test while the patient was present. Most clinicians sent blood specimens for HIV (79%) and syphilis (64%) confirmation but only 18% routinely sent cultures for gonorrhoea. The majority of clinicians (74%) practiced syndromic diagnosis for STDs/HIV. Among all, lack of availability of POCTs (43%), increased patient wait time (25%), and lack of training to perform the test (21%) were leading barriers to scale up POCTs. Comparing those who use syphilis POCTs to those who do not, a interruption of work flow was the greatest barrier identified (20% versus 12%, p=0.042). 60% of clinician participants felt that having increased access to POCTs for STIs would be useful.

Conclusion Increase of POCT availability and training could improve uptake of POCTs for STIs in Uganda and decrease the need for syndromic management which may lead to overtreatment and emergence of antibiotic resistance.

P1.06 IN SILICO MULTILOCUS SEQUENCE TYPING OF *CHLAMYDIA TRACHOMATIS* PLASMIDS SHOWS CLUSTERING OF ISOLATES ACCORDING TO THE DISEASE RELATED BIOVARS

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Introduction Nucleotide sequencing of the *ompA* gene, encoding the outer membrane protein MOMP, divides *C. trachomatis* into 15 main genovars comprising three biovars associated

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