26TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE & CLINICAL AND PUBLIC HEALTH WORKSHOPS

PART III - FINAL

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Abstracts selected by Mauro Romero L Passos, Felipe DL Passos, Marc Steben

Human papillomavirus (HPV) is the most frequent sexually transmitted infection causing 5% of all cancers in human. HPV is the source of various ano genital genital such as cervical, anal, vulvar, vaginal, penile as well as of oropharyngeal cancers. HPV also causes non neoplastic anogenital and respiratory track papillomatosis. Both high risk and low risk HPV now represent, at least partialy, preventable burden on the health care system and global loss of productivity.

The 26th International Papillomavirus Conference and its two workshops were held under the theme of "Sharing Knowledge for Global Health". Four commitments were made by the local organizing committee and we made sure to keep them to the International Papillomavirus Society: 1) to foster integrative research, 2) to enhance developing countries' capacities, 3) to inspire young researchers and 4) to offer an affordable learning experience.

There were 1978 participants at the conference. Two 2-day Workshops, Public Health Workshop & Clinical Workshop, preceded the main conference. During the conference we had 12 plenary sessions, 29 parallel Oral Communications Sessions, eight Early Morning Workshops (for young researchers and addressing capacity building in emerging countries), 18 Satellite Symposia (12 academic and 6 industries sponsored), 642 regular Posters and 70 e-posters.

The organisers followed very consistently an environment-friendly policy avoiding printed paper documents, increases the distribution of CD-ROM with all conference abstracts, free webcast dissemination of the presentations and the book of abstracts trough www.hpv2010.org. To recognize the strength and the variety of researchers in our society, awards were given for both oral and poster presentation in all of our four group of interest: basic sciences, clinical and laboratory sciences, epidemiology and public health as well as capacity building. Awards for young researchers and from developing countries were also given. The current paper contains short summaries of award recipients. Readers interested in the details of certain sessions or presentations are invited to consultwww.hpv2010.org.

Held under the theme: sharing knowledge for global health, the conference continues the tradition started last year in Malmo where every presentations were made available free to anyone joining the website, after an identification process, anyone can look or download presentations from the conference or the clinical or the public health workshop. This generosity from our speakers makes it possible to access the high quality of science and methods to all and not only to those able to make it to Montréal. This certainly helped us guarantee that in fact we delivered on our theme of sharing knowledge for global health and this unique collaboration with the Brazilian Journal of STD is a vibrant show of the interest our brazilian colleagues have spread the knowledge on HPV to their scientific community of Brazil and the editor has to be applauded for his vision and help in sharing the highest quality of knowledge!

Obrigado,

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P-101: The First Complete Papillomavirus Characterized from a Marsupial Host, the Bettongia Penicillata Papillomavirus Type 1

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Papillomaviruses (PVs) have been found in numerous mammal species, all of which however were placental mammals. We report the first papillomavirus found in a marsupial host, the Bettongia penicillata papillomavirus type 1 (BpPV-1). BpPV-1 was found in a papillomatous lesion of a brush-tailed bettong (Bettongia penicillata) living in Western Australia. Total DNA was extracted from a frozen skin biopsy of this lesion and used as a template for multiply primed rolling-circle amplification (RCA). The RCA product was cut using the EcoRI, XbaI and HindIII restriction enzymes, subsequently cloned and the complete genomic sequence was determined. The BpPV-1 genome contains 7737 bp and codes for 5 early open reading frames (ORF), E6, E7, E1, E2 and E4, and 2 late ORFs, L2 and L1. Phylogenetic analyses of a concatenated alignment of the E1, E2, L2 and L1 ORF of BpPV-1 and 62 other PVs revealed that BpPV-1 clusters close to the root of the phylogenetic tree. Pairwise alignment revealed that the L1 and L2 ORFs are most similar to those of bandicoot papillomatosis carcinomatosis virus types 1 and 2 (BPCV) isolated from the marsupial hosts Perameles bougainville and Isoodon obesulus, respectively. These viruses have an early region similar to the early region of polyomaviruses and a late region similar to the papillomavirus late region. In a phylogenetic tree based on a concatenated alignment of the L2 and L1 ORF of BpPV-1, the 2 BPCVs and 62 other PVs, BpPV-1 also clusters together with BPCV-1 and BPCV-2. BpPV1 is the first fully sequenced PV found in a marsupial host. These sequence data provides support to the hypothesis that the BPCVs arose by genetic recombination between an ancient PV and an ancient polyomavirus.

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P-102: Novel Equine Papillomavirus Clusters only Roughly with Other Equine Papillomaviruses

Christian Lange[1,2], Kurt Tobler[2], Mathias Ackermann[2], Claude Favrot[1]

The number of described papillomavirus (PV) species and potential PV genera is steadily increasing. It seems that every host species might potentially harbour a large set of PVs, but the ensemble of PVs from each species appears to belong only to a few genera. This has been observed for more than hundred human, but also for ten bovine and recently for seven canine PVs. In most of the other host species too few PVs have yet been identified to consider this hypothesis. We identified the DNA of a novel equine PV in aural plaques of a horse. The genome of a novel PV was amplified from a skin sample and was entirely cloned and sequenced. The novel equine PV genome contains the characteristic open reading frames (ORFs) E6, E7, E1, E2, L1 and L2, a large non coding region between the late and early region as well as a small non coding region between the early and the late region. The novel virus was consequently designated as equine PV3 (EcPV3). The genomes of the three equine PVs were analysed and compared with each other and further PVs. Upon phylogenetic analyses the equine PVs group well together and appear in pylogenetic vicinity to some bovine PVs. Still the three equine PVs share less than 60% of nucleotide identities in L1, and may therefore be regarded as belonging to different clades. Pairwise alignment of the L1 amino acid sequences reveals that FdPV3 shares only 56.3% identities with EcPV1 and 54.9 with EcPV2. These findings may support an allocation of the equine PVs into some distinct clades, as seems to be the case in other species.

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P-103: Anake Papillomavirus Does not Cluster with Other non Mammalian-Papillomaviruses

Christian Lange[1,2], Kurt Tobler[2], Jessica Gull[3], Mathias Ackermann[2], Elisabeth Vetsch[1], Claude Favrot[1]

In the past decades, papillomaviruses (PVs) infecting mammalian species have been discovered in a broad genetic diversity, forming a steadily growing number of genera. The more recent discovery of PVs infecting non-mammalian species extended the potential hosts to the whole clade of amniotes. Still only four whole genomes of PVs from non-mammalian hosts have been published as yet. We discovered PV DNA in pigmented papilloma-like lesions of a diamond python (Morelia spilota spilota). The genome of a novel PV was amplified from one of these lesions, was cloned and sequenced. It contains the characteristic open reading frames (ORFs) E6, E7, E1, E2, L1 and L2, a large non coding region between L1 and E6 as well as a small non coding region between E2 and L2. The size of the viral genome is, with only 7048 nucleotides, rather small, which is also the case in the two other published reptile PVs. A phylogenetic analysis based on the nucleotide sequence alignment of the L1 ORF placed the Morelia spilota papillomavirus1 (MsPV1) close to the root. Interestingly MsPV1 does not cluster with the other four PVs of sauropsids. Pairwise alignment of the L1 sequences on the amino acid level revealed only 42.9% and 42.5% identities with the described sea turtle PVs (CmPV1, CCPV1) as well as 43.9% and 43.6% identities with the two bird PVs (FcPV1, PePV1). Pair wise alignment with other L1 sequences revealed highest identities with the polar bear PV (UmPV1 - 56.5%) and two human PVs (HPV9 - 56%, HPV1 - 55.5%). These findings suggest that mammalian and sauropsid PVs may not be as genetically distinct in general as could be anticipated based to the previous findings.

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P-104: Novel Cetacean Papillomaviruses Exhibit both Chimeric and Non-Recombinant Cenome Organizations

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Currently known cetacean papillomaviruses (Omicronpapillomavirus) appear as chimeras. They exhibit genetic elements of viruses either with the shared tropism to mucosal tissue such as Alphapapillomavirus (i.e., early genes) or isolated from related host species such as Xipapillomavirus (i.e., late genes). We isolated and sequenced the complete genomes of four novel papillomavirus types and one variant from genital and oesophageal lesions of cetaceans, namely of Delphinus delphis (DdPV), Lagenorhynchus acutus (TtPV-3 variant) and Phocoena phocoena (PpPV-1, PpPV-2 and PpPV-4). Phylogenetic analyses were performed using Maximum Likelihood and Bayesian approaches. As inferred from early genes analyses, cetacean papillomaviruses, including the new types and variant, constituted a monophyletic group with (mucosotropic) Alphapapillomavirus. Analyzing late genes, they were the closest relatives of (ruminant) Xipapillomavirus, with the exception of PpPV-4. This was the unique cetacean papillomavirus that showed a consistent phylogenetic position close to the primarily mucosotropic clade. An explanation for incongruent tree topologies between early and late genes phylogenies are recombination events. PpPV-4 is the only known non-recombinant cetacean papillomavirus type and may represent a relative of a donor to chimeric Omicronpapillomavirus. Our study thus supports a complex evolutionary scenario with multiple driving forces for papillomavirus diversification, also including interspecies transmissions.

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P-105: Novel monkey betapapillomavirus associated with hand and foot papillomas

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Betapapillomaviruses are a diverse genus of papillomaviruses (PVs) commonly associated with human skin papillomas and epidermal dysplasia. Few beta-PVs have been characterized in nonhuman species. This report describes a novel beta-PV, named Macaca fascicularis PV type 2 (MfPV-2), isolated from exophytic skin papillomas on the hands and feet of an adult male cynomolgus monkey (M. fascicularis). On histology the papillomas were composed of thickened epidermis with superficial foci of cytomegaly and cytoplasmic pallor. Nuclei exhibited moderate pleomorphism with frequent central clearing and peripheral chromatin. Positive immunostaining for PV antigen, p16, and the proliferation marker Ki67 was present multifocally within all layers of affected epidermal cells. Complete sequence identity (100%) was noted between PV genomes sequenced from hand and foot lesions. The MfPV-2 genome was 7632 bp in length and included putative open reading frames for E1, E2, E4, E6, E7, L1, and L2 genes, similar to other beta-PVs. The closest PV relatives to MfPV-2 are HPV115 (67.4% pairwise identity) and MfPV-1 (67.3% pairwise identity), which was also isolated from hand and foot papillomas in a cynomolgus macaque. Phylogenetic analysis placed MfPV-2 in a new species group (number 6) among the beta-PVs, distinct from MfPV-1. These findings provide further support for the idea that tissue tropism among ancient primate PVs developed prior to divergence of human and nonhuman primate PV types.

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P-108: Sulindac Inhibits Proliferation and Induces Apoptosis in Cervical Cancer Cell Lines

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Background: Sulindac, a commonly used NSAID, has been investigated as a potential novel therapeutic for different forms of cancer, including colon and breast. A recent study looked at the molecular action of this drug on the HPV18 infected cervical cell line, HeLa, and demonstrated that not only could it induce apoptosis but also degrade one of the main oncoproteins, E7.
Design: This study aimed to validate the previous findings and to extend the analysis to other cervical carcinoma cell lines with differing origins, HPV status and viral DNA content. Three cervical cancer cell lines were examined, the adenocarcinoma derived HPV18 positive HeLa, the squamous cell carcinoma derived HPV16 positive SiHa and the HPV negative C33A.
Results: Sulindac had a time and dose dependent growth inhibitory effect on all three cell lines. However, the most potent response was observed in the HeLa cells, with the IC50 value approximately 200 μM less than the other two cell lines. Analysis of the HeLa cells demonstrated that this activity occurred predominantly through the induction of apoptosis but additionally by cell cycle arrest. The previous findings that sulindac induced a post-transcriptional degradation of HPV18 viral oncogene E7 were validated. This decrease was dose dependent and appeared to correlate with an observed G1 arrest. However, in comparison with the previous findings these results were observed with significantly lower concentrations of sulindac, 115 μM compared to 500 μM. In addition, it was demonstrated that a decrease in COX activity may

be partially responsible for the anti-proliferative activity of sulindac. **Conclusion:** This data indicates that the antineoplastic activities of sulindac are multifaceted. Since most cancers progress through the action of multiple pathways, drugs that simultaneously block several pathways might be particularly effective as therapeutic agents. Therefore, these results suggest that NSAIDs may offer potential as novel therapeutics for cervical cancer.

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P-110: In Vitro Selection of RNA Aptamers to HPV-16 L1 VLPs

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Cervical cancer is causally associated to persistent infection with high risk human papillomavirus (HPV), especially HPV-16. The HPV capsid is mainly composed of the L1 protein that can self-assemble in the absence of genetic material forming virus-like particles (VLPs) that are structurally and immunologically similar to the infectious virions. Aptamers are oligonucleotide ligands obtained by the iterative application of the SELEX method (Systematic Evolution of Ligands by EXponential enrichment) over combinatorial libraries and are capable of binding tightly and specifically to its targets. In this work, aptamers that specifically recognize HPV-16 virus-like particles (VLPs) were isolated from a combinatorial library of 415 variants using a modified SELEX method. Several aptamers were obtained after three cycles of negative selection and five cycles of positive selection against HPV-16 L1 VLPs. These aptamers were sequenced and divided into three groups according to sequence similitude. An aptamer representative of each group was probed for specificity against HPV-16 VLPs, the selection system and bovine albumin. Although all the probed aptamers efficiently bound the VLPs, the Sc5-3 aptamer showed the highest specificity. Later experiments using bacteria-produced HPV-16 L1 and GST-L1 chimeras resulted also in specific binding by Sc5-c3 aptamer, suggesting that recognition of L1 is not dependent in quaternary structure. Current experiments are aimed to establish the L1 domain associated to Sc5-c3 binding and cross-reactivity against other HPV types. Highly specific L1-recognizing aptamers have great potential as diagnostic tools and as antiviral agents to block HPV-16 infection.

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P-112: Human Papillomavirus 6/11-Specific T-cells Binding Class II Tetramer-E2/E6 Complexes elicit reduced IFN-?

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Objectives: The HLA-DRB1*0102, DRB1*0301 haplotype is associated with increased severity of RRP, a disease caused by HPV-6/11. TH1-like responses to E6/E2 proteins have been shown to protect against papillomavirus-induced disease in animals, and TH2 polarization may be related to RRP disease severity in DRB1*0102 and DRB1*0301 expressing patients with RRP. **Methods:** Using tetramer guided epitope mapping, we identified the dominant immunogenic peptides within HPV-11 early proteins E6/E2, restricted by DRB1*0102 and/or DRB1*0301. Peptide binding, tetramer staining, and proliferation assays identified minimal epitopes within these peptides. The cytokine profile of sorted tetramer positive T-cell lines from RRP patients (n=6) and controls (n=10) was measured using a cytokine capture assay, to determine E2/E6-responsive T-cell polarization. **Results:** Two distinct E6/E2 peptides (E6 113-132, E2 1-20) contained DRB1*0102 and DRB1*0301 restricted epitopes respectively. An additional peptide (E2 281-300) contained an epitope presented by both DRB1*0102 and DRB1*0301. Minimal epitopes within these peptides bound to recombinant DR protein, gave positive tetramer staining for sorted T-cell lines, and elicited T-cell proliferation in both RRP patients and HLA-matched healthy controls. While the magnitude of responses to these epitopes was similar in both groups, IFN-γ secretion was substantially lower in T-cell lines isolated from RRP patients and IL-13 was expressed by some tetramer-peptide binding T-clones. **Conclusions:** CD4+ T-cells specific for E2/E6 epitopes are easily detected in RRP patients and healthy controls with DRB1*0102 and DRB1*0301 haplotypes. However, RRP patients exhibit HPV-specific, immune dysregulation, indicated by decreased IFN-γ, but increased IL-13 expression. Therefore, therapeutic vaccination or other interventions that repolarize T-cell responses and restore TH1-like cytokine responses to HPV proteins could improve outcomes for RRP patients.

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P-114: Reversal of HPV-Mediated Suppression of Langerhans Cell Function with the TLR3 Agonist PolyICR

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Background: The HPV family of viruses establishes persistent infections because it has evolved mechanisms that allow it to evade the human immune system. Studies from our laboratory have identified HPV-mediated suppression of antigen presentation by Langerhans cells(LC) as a key mechanism through which HPV evades immune surveillance. PolyICR is a stable TLR3 agonist that is a broad inducer of innate immunity and is being developed as a vaccine adjuvant and antitumor agent. An important feature of PolyICR is its ability to enhance dendritic cell expression of cell surface markers, cytokine production and functional activation of T cells.

Objectives: In this study, we determined whether PolyICR can overcome HPV-induced immune suppression by functionally activating LC in the presence of HPV16 and inducing activation of HPV16-specific T cells.
Methods: Human LC were analyzed for the expression of MHC and T-cell co-stimulatory molecules, production of Th1 inducing cytokines, in vitro migration, and activation of HPV16-specific T cells when LC were exposed to HPV16 VLP and subsequently to PolyICR.
Conclusions: PolyICR was able to activate LC that had been pre-exposed to HPV16 VLP such that expression of MHC, CD40, CD80, CD86, and CD83 were highly upregulated and LC secreted high amounts of Th1 and inflammatory cytokines and chemokines. Upregulation of the chemokine receptor CCR7 resulted in a significant increase in migration capacity. LC incubated with HPV16 VLP and treated with PolyICR induced an HPV16-specific CD8+ T cell response detected by interferon gamma Elispot and MHC tetramer analysis that was absent when LC were exposed to VLP alone. These data suggest that the TLR3 agonist PolyICR is a promising therapeutic molecule that can overcome HPV-induced immune suppression of LC and result in an LC capable of stimulating an anti-HPV T-cell mediated immune response.

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P-115: Detection of Human Papillomavirus Type 16 E7-Specific T Cells by Elispot Assay

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Objectives: The study aims to evaluate the effect of a human papillomavirus type 16 (HPV16) E7 synthetic peptides on the antigen-specific T-cell response in Carcinoma in situ (CIS) and cervical cancer patients.
Methods: We characterized the HPV-16 E7 specific T-cell epitopes using E7 overlapping peptide pools with peripheral blood lymphocytes obtained from normal healthy donors and HPV-16+ 5 CI patients and 3 invasive cervical carcinoma patients with informed consent. We then analyzed the difference in the HPV-16 E7-specific T-cell immune responses in patients during or after treatment of the lesion by ELISPOT assay.
Results & Conclusions: All of CIS patients were underwent loop electrosurgical excision procedure (LEEP) and all of cervical carcinoma patient were type III radical hysterectomy. Analysis of peripheral blood lymphocytes obtained from patients with HPV-16+ CIS and cervical carcinoma showed that the HPV-16+ E7 peptide pool 2-3 (aa 16-55) specific CD4+ T-cell immune response was significantly higher than other peptide pool. The HPV-16 E7 peptide specific T-cell immune response correlates with regression of established HPV16+ lesions and freedom from disease recurrence. Thus, this E7 epitope may be useful for the characterization of HPV-specific immune responses in patients infected with HPV-16 or immunized with HPV vaccines.

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P-117: Cellular Immune Response to Human Papillomavirus 58 L1, E6 and E7 in Chinese Women with Transient Infections and Cervical Neoplasia

Paul Chan[1], Shih-Jen Liu[2], T.H. Cheung[1], Winnie Yeo[1], SM Ngai[1], Jo Cheung[1], Stephen Man[3]

Background: A relatively high prevalence of HPV58 has been observed among invasive cervical cancers (ICC) from certain parts of the world, especially the eastern Asia, where a vaccine targeting specifically against HPV58 may be needed.
Subjects and Methods: T-cell responses to HPV58 L1, E6 and E7 peptides among Hong Kong Chinese women with transient infections, cervical intraepithelial neoplasia (CIN) grades 2 and 3, and invasive cervical cancers (ICC), were examined. Overlapping 9-mer peptides were screened using an in-vitro peptide binding assay, and followed by an interferon-γ enzyme-linked immunospot (IFN-γ ELISPOT) assay using peripheral blood mononuclear cells (PBMCs). Altogether, 91 women with HPV58 infection; including 32 transient infections, 16 CIN2, 15 CIN3, and 28 ICC, were examined.
Results: L1 peptides located at amino acid positions 296-304, 327-335, 101-109 and 469-477 showed positive IFN-γ ELISPOT results from 4 HLA A11 subjects, and the responses were mainly observed from women with transient infections. On the other hand, responses to E6 and E7 peptides were mainly observed from subjects with CIN2 or above. Two HLA A11-restricted E6 peptides (amino acid positions 64-72 and 94-102), and 3 HLA A11-restricted E7 peptides (positions 78-86, 74-82, and 88-96) showed positive responses. One HLA A2-restricted E6 peptide located at the position 99-107 had elicited positive responses from 2 CIN2 subjects. Another HLA A24-restricted L1 peptide located at position 468-476 was found to have positive responses from 2 CIN2 subjects.
Conclusions: T-cell responses were of low intensity and only detected in a small proportion of infected subjects across different grades of cervical lesion. The E6 and E7 epitopes identified in this study can be considered for further investigation for their role as therapeutic vaccines against HPV58-associated cervical neoplasia.

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P-118: Cellular Immune Response to Human Papillomavirus 52 L1, E6 and E7 in Chinese Women with Different Grades of Cervical Lesion

Paul Chan[1], Shih-Jen Liu[2], Jo Cheung[1], T.H. Cheung[1], Winnie Yeo[1], Stephen Man[3]

Background: HPV52 has been found in a relatively high prevalence among cervical neoplasia in East Asia. Vaccines target specifically at HPV52 may be required for this region. The E6 and E7 proteins encoded by HPV are prime targets for therapeutic vaccine development.
Subjects and Methods: 95 Hong Kong Chinese women with

confirmed HPV52 infections; including 33 transient infections, 17 CIN 2, 15 CIN3, and 30 invasive cervical cancers had peripheral blood mononuclear cells collected to measure the T-cell responses using interferon-γ enzyme-linked immunospot (IFN-γ ELISPOT) assay.
Results: Epitopes located at amino acid positions 103-111, 332-340, 342-350 and 373-381 of the L1 protein; and at 27-35 and 86-94 of the E6 protein; and at 1-9 and 27-35 of the E7 protein, were found to elicit positive responses from HLA A11 individuals. A24-specific epitopes included 60-68 and 98-106 of the L1 protein, 42-50 and 59-67 of the E6 protein, and 24-32 of the E7 protein. One epitope (99-107) of the E6 protein showed positive responses for HLA A2 subjects.
Conclusions: The proportion of subjects showing detectable T-cell responses and the intensity of responses were low across all grades of cervical neoplasia suggesting that immune evasion mechanisms had set on early in the course of disease progression. T-cell responses against L1 were mainly confined to subjects who had recently cleared an HPV52 infection, whereas E6- and E7-specific responses were mainly observed from subjects who had developed cervical neoplasia. Further studies are warranted to evaluate the usefulness of these epitopes as therapeutic vaccine candidates.

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P-119: Sequence Variations of Human Papillomavirus Type 58 E2, E4 and E5 Genes Across the World

Paul Chan[1], Jo Cheung[1], Alfred Luk[1], Wendy Ho[1]

Introduction: HPV58 is more common in Asia and ranks the second or third among cervical cancers from Chinese populations.
Method: A total of 571 HPV58 samples, including 521 cervical scrapes and biopsies, and 50 penile scrapes collected form 15 countries were sequenced for the full length of E2, E4 and E5 genes. The phylogenetic relationship among different HPV58 variants was analysed by constructing maximum likelihood (ML) phylogenetic trees using PAUP* 4.0 b10.
Results: A total of 410 isolates were successfully sequenced for the E2 and E4 gene, and 421 isolates for the E5 gene. 76 variants of E2 were identified, but none was identical to the prototype. E2-WW01 and E2-WW02 were found in 43.9% and 16.8% of the specimens, respectively. Nucleotide positions 3445, 2935, 3685, 3571 and 2932 were the five most variable sites. The ML tree had a stem-like pattern with a main stem passing through variants E2_WW01, E2_WW39, E2_WW76, E2_WW02, and E2_WW07. 36 variants of E4 were identified, but none was identical to the prototype. E4-WW01 and E4-WW02 was found in 50.5% and 18.8% of specimens, respectively. Nucleotide positions 3445, 3571, 3596, 3411 and 3550 were the five most variable sites. No significant cluster was observed in the ML tree. 47 variants of E5 were identified. The prototype-like variant (E5_WW18) was found in only 1% of isolates. E5-WW01 and E5-WW02 was found in 48.2% and 22.1% of specimens, respectively. Nucleotide positions 3949, 3988, 3957, 4047 and 3930 were the five most variable sites. Only one significant cluster was observed in the ML tree, which only contained 2 variants.
Conclusion: Nucleotide sequence variations occur in the E2, E4 and E5 genes; but the variations are not sufficient for HPV58 lineage grouping. Clinical and epidemiological implication of these sequence variations remain to be established.

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P-120: Correlation of Mucosal Type 1 Immune Response to Oncogenic Human Papillomavirus (HPV) in Cervical T Cells to Fate of Precursor Lesions of Cervical Cancer

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Introduction: Given that the cervical intraepithelial neoplasia (CIN) develops in the cervical mucosa followed by human papillomavirus (HPV) infection, the mucosal cytotoxic (type1) immunity against HPV is necessary for control of CIN. The aim of this study was to establish a method for measuring mucosal anti-HPV cellular immune response and to examine the relationship between the mucosal immunity and the CIN course. In this study, we focused on mucosal type1 immune responses to HPV16 E7 oncoprotein.
Patients and Methods: Cervical lymphocytes (105-6 cells) were obtained from cervix of 27 patients with CIN lesions by Cytobrash under written informed consent. Among them, 17 patients with CIN 2-3 were positive for HPV16, and 10 patients with CIN 1/2 were positive for other HPV ("Other HPV group"). Seventeen patients with CIN 2-3 were grouped according to their course; 8 patients who received conization after sampling due to progression were classified into "Cone group", and 9 who dispensed with conization after sampling due to stable diseases or regression to CIN1 into "Treatment-free (TF) group". Cervical lymphocytes were examined for ELISPOT assay to detect HPV16 E7-specific IFN-gamma producing cells. Numbers of E7-specific IFN-gamma producing cells (anti-E7 type1 immune cells) were compared between the three groups.
Result: FACS analysis revealed that 3.7-20% (median=6.0%) of the cervical lymphocytes expressed CD3 (CD4:CD8=6:4) whereas 0.1-0.5% expressed CD19. Number of anti-E7 type1 immune cells of TF, Cone and Other HPV groups was 63-126 (average=98.6), 0-70 (average= 36.0), and 0-71 (average=42.7) /104 cells, respectively. The cell number of TF group was significantly higher when compared to either Cone or Other HPV group (t test; $p<0.005$).
Conclusion: Patients who induced mucosal E7-specific type1 immune responses can control CIN2-3 lesions spontaneously. The number of anti-E7 type1 immune cells in the cervical mucosa was a promising-biomarker to determine a fate of CIN2-3 lesions.

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P-121: Cytokine Levels in Cervicovaginal Secretions of Women with High Squamous Intraepithelial Lesions (HSIL)

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Background: Current data shows that human papillomavirus (HPV) is the primary causal agent in the development of cervical cancer. Most HPV infections likely involve a balance of Th1 and Th2 type immune responses.

Objectives: To measure cervical levels of the interleukin (IL)-4, IL-6, IL-8, IL-10 and IL-12 from women with HSIL and from women in a control group

Methods: A total of 40 women with a histopathological diagnosis of HSIL and 38 women without lesions but with high-risk HPV attended in Botucatu Medical School, and Amaral Carvalho Hospital, Jaú, SP. During the speculum exam cervical secretion was collected. The concentrations of the cytokines were determined using quantitative ELISA kits (R&D Systems). The research and genotyping assay of the HPV, in extracted secretions, was carried out on fragments of cervical biopsies obtained through a colposcopic examination of the cervix, using the L1 consensus PCR (GP5+/GP6+) and multiplex PCR.

Results: The median age of the control group was 33 years (22-60) and for the patients with HSIL, 37 years (19-70). Sixty patients (77.0%) were of Caucasian origin, 38.5% of the women were smokers and 23.0% reported lifetime monogamy. Of the women who were sexually active, the median of sexual intercourse was 1 in the control group and 2 in the HSIL group. In relation to oral contraceptives, 44.9% of all patients reported having used. DNA-HPV was detected in all cervical biopsy samples and the most common HPV types identified were, in order of decreasing prevalence, HPV-18, -33 and -16. No significant difference in local IL-4, IL-6, IL-8, IL-10 and IL-12 was found in patients with HSIL compared to the control group.

Conclusions: The production of cytokines in the cervicovaginal secretions is similar in patients with HSIL and patients without lesions contaminated with high-risk HPV. Financial support: FAPESP: 2008/58861-3.

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P-123: IFN-?, IL-12, IL-18 and HPV in Cervical Carcinogenesis

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Background: The host response to malignant tumors is a primary function in cellular immunity, modulated by Th1 cytokines. Objective: To determine RNA expression of IFN-γ, IL-12, IL-18 in premalignant lesions and cervical cancer associated with HPV infection.

Methods: Were analyzed 123 biopsies with diagnosis of low grade squamous intraepithelial lesion (LSIL), 40 with high grade squamous intraepithelial lesion (HSIL), 48 of cervical carcinoma (CC) and 66 cone fragments obtained from women with colposcopy findings of HPV infection and/or cervical lesion. Thirty-tree cervical scrapes from cytologically normal women (CN) were studied too. PCR was used for HPV screening with the MY09/MY11 and GP5+/GP6+ primers; RFLPs or sequenciation were used for typing and cytokines mRNA expression was detected by RT-PCR.

Results: The prevalence of HPV infection was 83% in LSIL, 77.5% HLSIL and 100% in CC (**Table 1**). The most common types viral were 16, 18, 58 and 31. IFN-γ mRNA was found in 27.6% of the CN samples, 3.4% of LSIL, 15% of HSIL and 39.4% of CC. The SIL and CC samples that expressed IFN-γ had high oncogenic risk HPV DNA; 48.5% of CC expressed IL-18 mRNA, and the level of transcripts was higher in CN than in CC; the lowest levels were found in SIL. Only 65% of IL-18 positive biopsies were HPV-positive of which 43% were HPV 16-positive. None of the samples with SIL or CC expressed the message of IL-12p35 and p40 subunits and only 25% of the CN samples had IL-12p35 mRNA. Only 48% of the samples were IFN-γ and IL-18 mRNA positives (**Table 2**).

Table-1. Infection by HPV in cases of SIL and CC, detected by PCR-MY and PCR-GP+. State of the Guerrero, Mexico, 2003.

	Infection by HPV				
PCR -MY PCR-GP+	Negative Negative	Negative Positive	Positive Positive	Positive NR*	Total
	n (%)	n (%)	n (%)	n (%)	n
LSIL	21 (17)	34 (27.6)	52 (42.3)	16 (13.0)	123
HSIL	9 (22.5)	5 (12.5)	11 (27.5)	15 (37.5)	40
CC	0 (0.0)	4 (8.3)	3 (6.3)	41 (85.4)	48
Total	30	43	66	72	211[a]

[a] The percentage calculated by column, the totals in column and row.
*It was not realized.

Table-2. Expression of the mRNA of IL-12 and IL-18 in samples with detectable levels of the transcripts IFN-γ, in cervical tissue with normal cytology, SIL or CC.

		mRNA IL-12p35^{pos}/ IL-18^{pos}	mRNA IL-12p35^{pos}/ IL-18^{neg}	mRNA IL-12p35^{neg}/ IL-18^{pos}	mRNA IL-12p35^{neg}/ IL-18^{neg}
mRNA de IFN-γ ^{positive} (n)					
CN	8	2/8	2/8	3/8	1/8
LSIL	4	0	0	1/4	3/4
HSIL	6	0	0	2/6	4/6
CC	13	0	0	9/13	4/13

Pos: Positive expression, Neg: Without expression

Conclusions: In cervical tissues infected by HPV there is important dysregulation in IL-18, IL-12 and IFN-γ, which are key in the Th1 activation response and favoring persistent HPV infection and increasing the risk to develop premalignant lesions as well as cervical cancer.

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P-125: Sequence Variation of Human Papillomavirus Type 58 E7 Across the World

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Introduction: HPV58, though rare worldwide, ranks the second or third among cervical cancers detected from Chinese populations. The E7 oncoprotein disrupts cell cycle regulation and is a key leading to the transformation of infected cells. **Method:** A total of 571 HPV58-positive samples, including 521 cervical scrapes and biopsies, and 50 penile scrapes collected form 15 countries were analysed. The E7 gene was sequenced and aligned by CLUSTAL X to identify HPV58 E7 variants. The entropy of each variable nucleotide position was calculated. The phylogenetic relationship among different HPV58 variants was analysed by maximum likelihood (ML) trees generated by PAUP* 4.0 b10, and Bayesian phylogenetic trees constructed by MrBayes v3.1.2. **Results:** A total of 441 isolates were successfully sequenced with 22 variants identified. The prototype-like variant being detected in 5.7% of isolates, was the forth most common variant found. E7-WW01 and E7-WW02 were found in 49.7% and 21.1% of the specimens, respectively. The second and forth most common variant, E7-WW02 and E-7-WW04, were found more commonly in Asia. Nucleotide positions 694, 760, 632, 793 and 801 were the five most variable sites, with an entropy value of 0.69, 0.57, 0.53, 0.40 and 0.39, respectively. All these variations involved amino acid changes. The 22 variants of E7 were divided into two main clusters in the ML tree, whereas three significant clusters were observed in the Bayesian tree. **Conclusion:** Multiple nonsynonymous sequence variations occur in the E7 gene of HPV58. It is worthwhile to further study the oncogenic implication of these variants.

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P-127: Determination of the Diagnostic Accuracy of Testing for High-risk (HR) Human Papillomavirus (HPV) Types 16, 18 and 45 in Precancerous Cervical Lesions

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Objectives: The purpose of this study was to determine the positive predictive value of HR-HPV testing for precancerous lesions of the cervix uteri. Therefore, we focused on the diagnostic accuracy of testing for one or more of the HPV types 16, 18 and 45 for all HR-HPV positive women. HR-HPV infections with subtypes 16, 18 and 45 have demonstrated a higher risk of developing cervical cancer [Bulk S, et al. Br J Cancer 2006; 94:171-5]. **Methods:** Between 2007 and 2008 a total of 586 women were recruited: a group of 477 women with a history of known cervical lesions and/or HPV infections (eligibility criterion: HR-HPVDNA positive test result with HC2T) and a group of 109 women who were examined as part of their routine cervical cancer screening. Baseline HR-HPV status was measured at enrolment with the FDA-approved Hybrid Capture® 2 HPVDNA Test and the HR-HPV 16/18/45 Probe Set Test (HC2T, PST; QIAGEN, Hilden, Germany). Cervical smears were classified according to the Second Munich Nomenclature (1989). The results were converted to the nearest equivalent in the Bethesda system. In general, study subjects were followed up semi-annually for a period of 11/2 years. The histopathological endpoint of CIN 2-3 lesion was used as a surrogate endpoint. **Results:** To date data for 43.5 % of the risk group and for the complete control group were available. CIN 2-3 was confirmed in 77 HR-HPVDNA positive women. 85.7% of these lesions were positive for one or more of the HR-HPV types 16, 18 and 45 (PST+). 88.2% (60/68) of the histologically confirmed CIN 3 lesions and six out of nine (66.6%) CIN 2 lesions were positive

PST+. Furthermore, all women with a histologically confirmed squamous cell carcinoma (n = 4) were PST+. Besides, three (50%) out of six detected CIN 1 lesions were PST+. Nonetheless, histology confirmed no malignancy in three cases. Two of them were PST+. Within the next few month study results will be updated. **Conclusion:** These preliminary results demonstrate that starting cervical cancer screening at the age of 20 years remains important as seventeen (25%) of the 68 histologically verified CIN 3 lesions arose in women who were younger than 30 years. Our data suggest that adding an HR-HPV test that detects one or more of the HR-HPV types 16, 18 and 45 in conjunction with cytology could help to identify women with an underlying cervical lesion who have an elevated risk of developing severe cervical lesions.

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P-132: E6 and E7/HPV58 Variants in Women with Normal Cytology from the Colombian Cohort

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Background: Human papillomavirus type 58 (HPV 58) is highly prevalent in women with normal cytology in the Colombian population. Also this type is highly prevalent in women with high grade squamous intraepithelial cervical lesions (HGSIL) and in women with invasive cervical cancer worldwide. There are few studies of E6 and E7/HPV 58 variants and some of them have shown an association of variants presence with a higher risk of HGSIL and cervical cancer. In Colombia there are no studies of E6 and E7/HPV58 variants in women with normal cytology. **Objectives:** To identify E6 and E7/HPV58 variants in cervical scrapes of women with prevalent HPV 58 infections in the Bogotá cohort. **Methods:** The E6 and E7 regions of 34 samples HPV 58 positives were amplified using the E6F1/E7R1 primers for the ORF E6 and E7P1/E7P2 primers for the ORF E7. E6 and E7/HPV 58 variants were detected using automated direct sequencing. The reference sequence of HPV 58 was used to compare sequences. **Results:** 27/34 samples amplified the ORF E6 and E7 (79.4%). Twenty one of these samples have the T307/A694/G744/A761 variant (77.8%), three samples have the T307/T632/G744 /A760 variant (11.1%), one sample has the A169/T307/A599/A694/G744 / A761 variant (3.7%), one sample has the T307/A694/G744/A761/ G763 variant (3.7%) and one sample has the T307/G744 variant (3.7%). None of the samples had the reference sequence of HPV58. **Conclusions:** Five different variants were identified in the samples analyzed: 3 previously identified in Asian population and 2 new variants (A169/T307/A599/A694/G744/A761 variant and T307/ A694/G744/A761/G763 variant). The follow up analysis of these variants will give important information about the role of these variants in the persistence of HPV 58 infection and in the development of cervical intraepithelial lesions.

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P-133: HPV-16 Intratype Variation in Women Attending Gynecological Outpatient Department During 2009 in Spain

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Objective: To detect nucleotide polymorphisms within the LCR, E6 and L1 genes of HPV 16 in general population of Northwest Spain. **Materials and Methods:** 2 protocols were carried out for HPV detection and genotyping in endocervical samples received from women attending a gynecological outpatient department during the year 2009: nested PCR MY09/11-GP5/6 (followed by DNA sequencing) in one Microbiology Unit and Amplicor HPVtest and Linear Array HPV Genotyping Test (Roche) in another one. HPV 16 was detected in 170 women. For identification of HPV 16 sequence variation, 761bp from the LCR-E6 region (Nt 7701-591) and 422bp from the L1 region (Nt 6599-7021) were amplified (Expand HiFi, Roche) as previously described (Xi LF, 2006. Wheeler, 1997) and sequenced (BigDye 1.1. Abi Prism 3100-Avant). All sequences from a sample were combined for the analysis. For the multiple alignment and the phylogenetic analysis the reference sequences European (E), Asian (As), African (Af1, Af2), Asian-American (AA) and NorthAmerican (NA) were included (Yamada, 1995). **Results:** In 145 cases (average age 35.7 years) sequences of the three regions were available. The distribution of HPV 16 variants was: 131/145 (90.3%) E lineage and 14/145 (9.7%) non E variants. Of the 131 E lineage sequences, 66 (50.4%) grouped into the E-350G cluster (52 European Patron E-P, 4 E-C109G, 4 E-G131G, 2 E-C188G, 1 E-m), 61 (46.6%) into the E-350T cluster (all of them E-P) and 4 (3.1%) into the E-350G/350T one (E-P). Among the non E variants there were 7 AA, 4 Af2, 2 Af1 and 1 NA strains. **Conclusions:** European lineage was the most prevalent HPV 16 variant. There was a homogeneous presence of E-350G and E-350T. No Asian Variant was found in the studied population.

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P-136: Spectral Phenotype of Oncogenic HPV-infected Exfoliative Cervical Cytology

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Human papillomavirus (HPV) is a sexually-transmitted infection associated with cervical cancer. Over 100 HPV types have been identified of which 13 are high-risk oncogenic. In unvaccinated women worldwide, the incidence of cervical cancer from HPV 16 and 18 will remain. Cervical cytology can be graded from normal (atypia-free) to low-grade (borderline nuclear abnormality/cervical intraepithelial neoplasia [CIN] 1) to high-grade (CIN2/3). Infrared (IR) spectroscopy is a non-destructive technique that allows the acquisition of a biochemical-cell fingerprint based on vibrational states of chemical bonds in the interrogated sample. Exfoliative

cervical cytology specimens (n=147) were retrieved, graded by a cytologist and HPV-tested/genotyped using Hybrid Capture 2 and the Roche HPV Linear Array. Additionally, the spectral signatures cervical cell lines C33A, HeLa and SiHa were examined. After washing, cellular material was transferred to low-E glass slides and interrogated using attenuated total reflection Fourier-transform IR (ATR-FTIR) spectroscopy; 10 spectra were acquired per sample. Given the complex nature of a resultant dataset consisting of thousands of variables (wavenumbers), we used multivariate analysis for data reduction and information retrieval. Principal component analysis coupled with linear discriminant analysis (PCA-LDA) gave a visual representation of the data (scores plot) and, identification of the wavenumbers and consequent biochemical entities responsible for segregation (loadings plot). Immortalised cell lines were readily distinguishable from each other. It was difficult to segregate categories of cytology associated with specific HPV infection types. However, in low-grade cytology infected with high-risk oncogenic HPV 16 or 18, it was possible to segregate women based those aged 20 - 29 y and 30 - 39 y. This suggests a spectral phenotype in exfoliative cervical cytology associated with transient versus persistent HPV infection.

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P-137: A Competitive Serology Assay Shows Protection against HPV Infection by Natural Titers in the Guanacaste Natural History Study

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Background: VLP-based HPV ELISA serology assays only partially predict immunity. A novel competitive Luminex Immunoassay (cLIA) has been developed by M. Esser and colleagues to specifically measure neutralizing antibodies against HPV types included in the quadrivalent vaccine. **Objective:** We evaluated whether cLIA measured immunity against subsequent HPV6, 11, 16, and 18 infections in a subcohort of women nested in the Guanacaste Natural History Study.
Methods: We tested women who were HPV6, 11, 16, and 18 DNA negative at baseline and had a subsequent persistent infection or type-related CIN2+ with one of these 4 HPV types, and half of women with a subsequent transient infection (n=369 total). We also tested a sample of women from the cohort stratified by sexual activity and genotype status (n=498). The cLIA results are compared to previous VLP-based ELISA results for HPV16 (other types to be presented).
Results: The reproducibility for HPV16 cLIA testing was very good (R-square 0.95). Only 77/867 women were seropositive by HPV16 cLIA (including 0/69 virgins) compared to 224/867 by HPV16 VLP ELISA ($p<0.001$). ELISA but not cLIA seropositivity increased with number of previous sexual partners ($p<0.001$). HPV16 cLIA positivity was associated with protection against subsequent infections or HPV16-associated CIN2+ (OR 0.31, CI: 0.11-1.03). No women developing HPV16+ CIN2+ were cLIA positive. HPV16 ELISA positivity showed weaker protection (OR 0.63, CI: 0.42-1.04) that increased with strong seropositivity (OR 0.43, CI: 0.22-0.85). In paired, mutually-adjusted analyses, cLIA seropositivity was correlated with strong ELISA seropositivity; but cLIA positivity was the important predictor of immunity.
Conclusions: Neutralizing antibodies developing from the natural humoral immune response against HPV can be measured. Detection of neutralizing antibodies against HPV16 is associated with significant protection against subsequent infection and disease; however, the cLIA assay has low sensitivity to measure previous exposure.

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P-139: Seroprevalence of Seven High Risk HPV Types in the Dutch Population

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Background and Objectives: HPV infections are one of the most common sexually transmitted diseases. In March 2009 the Dutch government decided to routinely vaccinate all 12 year old girls against HPV-16 and HPV-18 with the bivalent HPV-vaccine (Cervarix, GSK). Data about the seroprevalence of high risk HPV types in the Dutch population are scarce. In 2006/2007 a large serum bank was established by means of a cross-sectional population-based study (ISRCTN 20164309). The serological antibody levels against 7 high risk HPV serotypes will be determined in this serum bank from the prevaccination era using a multiplex immuno assay (MIA) as developed by the vaccine producer. The MIA is validated for measurement of specific antibodies against HPV type 16, 18, 31, 33, 45, 52, and 58.
Methods: The cross-sectional serosurvey consists of samples from men and women between 0-79 years of age, n=6386 (1). The specific antibodies against seven high risk HPV types will be determined in this serum bank with a fluorescent bead-based multiplex immuno assay with virus like particles (VLPs) for the seven HPV serotypes coupled to the microspheres (luminex technology).
Results and Discussion: The antibody levels against the 7 high risk HPV serotypes obtained with the MIA will be presented. This study will contribute to gain insight into incidence of high risk HPV infections in the Dutch population. It is important to study the effects of the routine vaccination and the catch-up campaign started in 2009. Furthermore, possible shifts in the seroprevalence of the high risk HPV types can be distinguished by comparing the antibody levels in a serosurveillance study established in 1995/1996, with the data from the 2006/2007 study.

1. van der Klis et al, Neth. J. Med., 2009, 67:301-8.

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P-140: Calibration of an HPV 6/11/16/18 Serology Standard to an Internal Standard and WHO 05/134 for HPV 16

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Background: A Food and Drug Administration (FDA) or World Health Organization (WHO)/National Institute of Biological Standards and Controls (NIBSC) Human Papillomavirus (HPV) 6/11/16/18 human reference standard is not available. A 30L reference standard comprised of sera from women vaccinated with GARDASIL® was created to measure the antibody response to HPV 6, 11, 16 and 18 virus-like particles (VLPs).
Methods: A protocol was written and performed to collect serum and plasma from women within 6 months following vaccination with the HPV 6/11/16/18 VLP-containing vaccine, GARDASIL. The sera were screened in a competitive Luminex immunoassay (HPV-4 cLIA) for antibody titers to neutralizing epitopes on the VLPs and 31 individuals with high antibody titers were selected for plasma donation. The pooled reference serum was calibrated to an internal African Green monkey (AGM) reference standard and the WHO 05/134 type 16 reference standard. A set of 81 samples from GARDASIL vaccinees and non-vaccinees were used in the concordance analysis to determine the relative potency of the human reference standard in milli-Merck units/mL (mMU/mL).
Results: The provisional potency of the reference standard was determined to be 1274, 1198, 3544 and 686 mMU/mL for types 6, 11, 16 and 18, respectively and 276 IU/mL for type 16. The serostatus agreements using the two different reference standards were 95.5%, 97.5%, 99.4% and 93.2% for types, 6, 11, 16 and 18 respectively.
Conclusions: Since this reference standard is from women rather than African Green monkeys it is more qualitatively and quantitatively similar to sera from subjects enrolled in clinical trials and samples from sero-epidemiology studies. The results from this potency calibration suggest that this reference standard can be used by multiple laboratories to help harmonize results from vaccine clinical trials and sero-epidemiology surveys.

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P-142: Development of a GuHCl-Modified ELISA for Measuring the Avidity of Anti-HPV VLP Antibodies

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Modified ELISAs estimate avidity through using various chaotropic agents and measuring the degree to which they disrupt the interaction between antibody and antigen. The theory behind the assay is the higher the avidity of an interaction the less susceptible it is to the effects of a chaotropic agent. The goal of this study was to develop a modified ELISA to measure the avidity of human papillomavirus (HPV)-specific antibodies raised after vaccination with HPV virus-like particles (VLP) and potentially in the context of natural infection. The HPV VLP used for coating antigens are complex, multimeric protein antigens and presumably also susceptible to the actions of the chaotrope. Therefore, a number of chaotropic agents, including urea, diethylamine, guanidine hydrochloride and ammonium thiocyanate were evaluated in the assay for their effectiveness in measuring avidity while not affecting the integrity of the plate-bound VLP. Guanidine hydrochloride (GuHCl) was selected as an ideal chaotropic reagent because it had a minimal effect on the integrity of the plate-bound VLP. The assay was then successfully applied to a subset of sera from Cervarix® recipients from the NCI Costa Rica trial (n = 50) collected at month 12 visit. Samples were diluted to give a target OD of 1.0 and a range of concentrations of guanidine 0.5 to 3.5 M was used. Overall, the assay was highly reproducible (overall CV = 3.6%) and captured a considerable range of antibody avidity indices (median = 2.78 M, IQR = 0.96-3.26 M). Therefore, a GuHCl-modified ELISA is an acceptable, low cost and high throughput method to measure avidity indices of HPV-specific antibodies that can be used within a clinical trial setting. Additional studies are warranted to address the potential role of antibody avidity in protection against infection.
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P-143: Low Avidity of HPV 16 Antibodies is Associated with Increased Susceptibility to Low - but not High-Risk HPV Infections

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Background: Assessment of low avidity antibodies has been used for differential diagnosis of acute vs. recent infections with viral, bacterial and parasitic agents. The low-avidity antibodies may, however, persist for a longer period in some individuals.
Objectives: We studied whether low avidity of human papillomavirus type (HPV16) IgG antibodies is associated with susceptibility to other HPV infections (indicated by corresponding seropositivity).
Methods: A cross-sectional study was performed on HPV16 seropositive pregnant Finnish and Ugandan women. Three hundred and sixty-seven HPV16 seropositive women were randomly selected from four groups: A) HPV16 seropositives only, B) HPV16 and HPV6/11 or C) HPV31/33 or D) HPV18/45 seropositivity. We tested for avidity of HPV16 antibodies using HPV16 virus-like particles in a modified ELISA.
Results: The overall prevalence of low avidity HPV16 antibodies among the HPV seropositive women was 18% (68/368). The proportions of low avidity HPV16 antibodies in the different groups were: A) 17% (28/160), B) 33.8% (23/68), C) 10.8% (9/84), and D) 14.3% (8/56). Women with the low-avidity HPV16 antibodies had an increased risk of being seropositive for low risk HPV types 6/11 (odds ratio, OR=2.4; 95% CI, 1.2-4.5). No increased risk of being seropositive for high-risk HPV types 18/31/33/45 was observed.
Conclusions: Our findings suggest that there are differences in

risk of being seropositive for low-risk and high- risk HPV types by low-avidity of HPV16 antibodies. Associated characteristics of host immunity could modify susceptibility to the different HPV types.

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P-144: Detection of Anti-HPV Serum Antibodies 3 to 12 Years after Detection of HPV DNA in Genital Samples from Young Women

Joseph Carter[1], Mary Colangelo[1], Shu-Kuang Lee[2], Laura Sycuro[2], Laura Koutsky[2], Denise Galloway[1,3]

Detection of antibodies to multiple HPVs in a single well using GST-L1 fusion proteins on a Luminex platform has not been well validated. This study investigates the association between HPV-type specific seropositivity and alpha HPV genital infection detected years earlier, and to assess antibody persistence. Female university students (n=285) who had previously participated in a cohort study in which they were tested for HPV DNA at 4 month intervals over an average of 3.9 years were recalled for a follow-up study. The median elapsed time between HPV DNA detection in the first study and the initial follow-up visit was 7 years (range 3 to 12). At follow-up, antibodies to 16 genital HPV types were examined using a multiplex binding assay. Associations were confirmed if women in whom HPV DNA was detected in the 1st study were significantly more likely to be seropositive for that type at follow-up compared with women in whom HPV DNA was not detected. Statistically significant associations between the type-specific detection of HPV DNA and antibodies were found for types 6, 16, 18, 33, 35, 39, 52, and 67. Antibodies to HPVs 11, 31, 51, 56, 59 and 68 were also detected more frequently among women in whom HPV DNA had been detected in the first study compared with women who consistently tested negative for that type but the associations did not achieve statistical significance. Only antibodies to HPVs 45 and 58 showed no relationship to previous DNA detection. Among women in whom HPV DNA had been detected there was considerable variation in the proportion of seropositive women. To more fully address the persistence of antibodies, we are testing sera from the first study using the multiplex assay to 16 HPV types. The results will be compared with the long-term follow-up seroprevalence to assess persistence.

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P-146: Transcriptional Regulation of E-Cadherin by HPV16 E6

Zarina D'Costa[1], Charles Matthews[1], Merilyn Hibma[1]

Background: E-cadherin, a cell surface adhesion molecule, is needed for the retention of Langerhans cells (LC), the primary antigen presenting cells in the skin. We have previously reported that the E6 oncoprotein of HPV16 is able to cause a 50 % decrease in the levels of cell surface E-cadherin in vitro. This decrease is further enhanced when cells are co-transfected with both oncoproteins of the virus, E6 and E7. The objectives of this study were thus to investigate the potential transcriptional regulation of E-cadherin by HPV16 E6.
Methods: Quantitative RT-PCR was used to compare the levels of E-cadherin mRNA in E6 and control cells. A luciferase-based reporter assay was used to determine the activity of the wild type and deletion constructs of the E-cadherin promoter, in cells with and without E6. Spontaneously immortalized epithelial cells, HaCaTs, were transduced with retroviruses expressing HPV16 E6. Protein lysates were collected from these cells and stained for transcriptional repressors of E-cadherin via western blotting.
Results: The mRNA levels of E-cadherin were found to be 0.61 times less in cells expressing E6 compared to control cells. The measured luciferase activity showed that the E-cadherin promoter is less transcriptionally active in E6 expressing cells than control cells. Protein levels of Snail, the main transcriptional repressor of E-cadherin, were found to be elevated in cells expressing E6 relative to control cells.
Conclusion: HPV16 E6 is able to regulate the cell surface adhesion molecule E-cadherin at a transcriptional level. This manipulation of E-cadherin levels correlates with decreases in LC numbers in HPV16 infected skin, thus representing an immune evasion mechanism by HPV16.

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P-147: Human Papillomavirus Associated Loss of E-Cadherin Expression is Conserved across all but the b Genera

Cheng Mee Leong[1], John Doorbar[2], Ingo Nindl[3], Han-Seung Yoon[4], Merilyn Hibma[1]

E-cadherin is a cell adhesion molecule expressed predominantly on epithelial cells. Surface expression of E-cadherin is crucial for normal tissue organisation and morphogenesis and its loss is frequently associated with cancer. In the epidermis, E-cadherin has an additional role in Langerhans cell (LC) retention by mediating adhesion between keratinocytes and LC. We have previously reported that E-cadherin expression is frequently lost from the membrane of basal and parabasal keratinocytes in human papillomavirus type 16 (HPV16) infected cervical lesions. The aim of this study was to determine the degree of conservation of E-cadherin down-regulation among HPV types from other members of the α7 genus and from representative types from other genera. Lesions infected with α, β, γ or μ types (including only low-grade lesions from high-risk types), were examined for E-cadherin following immunofluorescence staining. In normal tissues (n=16), E-cadherin expression was membranous and strong. E-cadherin was cytoplasmic or heterogeneous (i.e. membranous staining was absent from the majority of cells) in 11 samples infected with

high-risk α7 or α9 types. In cutaneous lesions (n=9) infected with low-risk types from α4, μ or γ genera, staining was heterogeneous or negative. Although the majority of α10-infected lesions (n=7) showed heterogeneous E-cadherin staining, two tissues exhibited membranous staining and significantly β-infected lesions had normal E-cadherin expression. The relationship between E-cadherin expression and LC was determined in all tissues following CD1a and E-cadherin double staining. In all tissues, LC were localised in areas where E-cadherin was expressed and were depleted from tissues with low E-cadherin expression. E-cadherin down-regulation by HPV therefore was not restricted to high-risk types or mucosa-infecting types but was also observed in low-risk and some cutaneous HPVs. There was a correlation between E-cadherin loss and LC depletion, supporting the hypothesis that repression of E-cadherin expression influences the immune microenvironment by mediating LC depletion.

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P-148: Correlation of Local Inflammatory Cytokines and HPV Clearance Induced by Cryosurgery

Chamsai Pientong[1], Praphatson Malat[1], Tipaya Ekalaksananan[1], Bunkerd Kongyingyoes[2], Bundit Chumworathayee[3]

Low-grade cervical intraepithelial neoplasia (CIN I) lesions are almost always the result of human papillomavirus (HPV) infection. Spontaneous resolution of CIN I lesions is common, whereas persistent HPV infection is the risk of cervical cancer development. This study aimed to investigate the mRNA level of IFN-g, IL-10 and TNF-a in women with histology confirmed LSIL after cryosurgery and to determine the role of cryosurgery intervention in increased immune activation. At first visit, cervical cells and biopsies were collected from 100 women with LSIL within 3 months after histological diagnosis and detected for HPV DNA. The HPV positive cases were divided into cryosurgery (performed at second visit) and control groups. At third visit, cervical cells and biopsies were collected from both groups 6 months after cryosurgery, whereas only cervical cells were collected 12 months after cryosurgery. The HPV DNA was detected using PCR with GP5+/GP6+ primers and the mRNA level of IFN-g, IL-10 and TNF-a were determined in cervical tissues using real time PCR. The result showed that HPV DNA was found in 60 cases and the most common HPV was HPV16 (60%). Cryosurgery was performed in 29 cases within 4 weeks after the first visit and 31 cases were control group. At first visit, mRNA of IFN-g, IL-10 and TNF-a were expressed in 93%, 93% and 97% of 100 cases, respectively. The mRNA level of IFN-g, IL-10 and TNF-a were increased in both groups. However, cryosurgery did not differently activate mRNA expression from control when it was performed within 4 weeks after the first visit. Almost of HPV persistence detected 12 months after cryosurgery were found in cases with decreased mRNA level of IFN-g. These results suggested that, after biopsy or cryosurgery, inflammatory cytokines, especially, IFN-g were increased and correlated to HPV clearance.

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P-149: Alteration of Plasma TH2-Chemokines in Patients with Recurrent Respiratory Papillomatosis (RRP) Treated with Celecoxib

David W. Rosenthal[1,2,3], James A. DeVoti[2,3], Bettie M. Steinberg[2,4], Alan Abramson[4], Vincent R. Bonagura[2,3]

Objectives: RRP is characterized by recurrent benign tumors of the respiratory tract, caused by infection with human papillomavirus 6 and 11 (HPV). The immunologic mechanism(s) that govern disease variation remain unresolved, but we have previously shown an increased expression of TH2-like chemokines, (CCL17, CCL18, and CCL22). In a small pilot trial (n=4) we have shown clinical improvement in patient severity scores with administration of a COX2-inhibitor, celecoxib. We hypothesize that TH2-like chemokines play a critical role in the immune response of patients with RRP, and the pathogenesis of disease can be altered by COX2-inhibition. **Methods:** Patients enrolled in a twelve month pilot trial of a COX2-inhibitor, celecoxib, were followed longitudinally during administration of celecoxib (n=3). Plasma samples were assayed by cytokine specific (CCL17, CCL18, CCL22) ELISA (DuoSet, R&D Systems) every 3 months during administration of celecoxib. Patients with RRP not enrolled in the pilot trial had multiple plasma samples assayed over a twelve month period as a control. **Results:** CCL18 decreased in all three patients during celecoxib administration, while CCL17 and CCL22 did not change over twelve months. Patients not in the celecoxib pilot trial maintained similar chemokine expression levels in the plasma over 12 months. **Conclusions:** These results support our previous studies that show RRP is a TH2-like disease, and suggests that the disease may be modified by alteration of the TH2-like chemokine CCL18. CCL18 has been shown to be altered by prostaglandin E2, the target for COX2 and CCL18 is produced by T-cells, dendritic cells, and alternately activated macrophages. This study examines the mechanism by which COX2 inhibition may alter the host's response to HPV.

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P-150: Select Micro-RNA's (miRNA's) are Upregulated in HPV-6 and -11 Induced Recurrent Respiratory Papillomatosis (RRP)

James A. Devoti[1], David W. Rosenthal[1,2,3], Jennifer Diaz[3], Alan Abramson[4], Bettie M. Steinberg[1,4], Vincent R. Bonagura[1,3]

Background: The transcriptional profile of papillomas is characterized by altered expression of immune response genes, and another group of genes that are frequently seen in cancers. Individual micro-RNAs (miRNAs) regulate a large number of target genes and more than one miRNA may regulate a specific gene, resulting in complex genetic regulation. To determine if miRNAs effect the altered transcriptome in RRP, we compared expression levels of a panel of 88 miRNAs from papilloma tissue, vs. unaffected, autologous laryngeal tissue. **Methods:** Papilloma tissue (n=12) and unaffected, autologous laryngeal tissue (n=12) were obtained after obtaining informed consent. In order to enrich miRNA, small RNAs were separated by size exclusion from total RNA (>5µg each sample) that had been extracted from pairs of tissues. The small RNA directed cDNA synthesis, and the cDNA was then screened for individual miRNA expression levels using a commercially available expression profiling assay (MAH-001; SABiosciences). **Results:** Four miRNAs were up-regulated, and 7 were down-regulated, utilizing a fold change greater than 4.0 in the data analysis. However only the upregulated miRNAs (miR-195, miR-21, miR-27a, and miR-26-b), which were from 5.7 to 8.5 fold increased, were also statistically significant (p= 0.009 to 0.001). **Conclusion:** We have identified 4 miRNAs that may contribute to the altered transcriptional profile present in actively growing papillomas from patients with RRP. All of these miRNAs have been reported to also be upregulated in a variety of cancers and also in non-malignant diseases. We are currently confirming these findings in a larger set of archived RNA. This report is the first demonstration of altered miRNA levels in RRP. Understanding the pathways that are impacted by these regulatory molecules may provide new insight into the disease process, and could potentially identify rationale targets for therapeutic intervention.

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P-151: The Altered HLA Class I and HLA-G Expression is Associated with IL-10 Expression in Patients with Cervical Cancer

Josefa Antonia Rodríguez[1], Constanza Gómez[3], Martha Lucia Serrano[1], María Mercedes Bravo[1], Alba Lucia Cómbita[1,2]

Although the infection with high risk HPV is an important risk factor in cervical cancer etiopathogenesis, there is increasing evidence suggesting that the ability to avoid immunosurveillance seems to be linked to transforming potential of HPV and to a rapid progression to cancer. A shift from a Th1 to Th2 cytokine profile and the subsequent expression of IL-10 has been associated with impairment of tumour immunosurveillance and progression of cervical cancer. In other cancer models, the IL-10 contribute to the impaired anti-tumour immune response by down-regulating HLA-I expression or by increasing HLA-G expression. To evaluate the contribution of these mechanisms in cervical cancer progression, we analyzed the association of HLA-I, HLA-G and IL-10 expression by immunohistochemistry in tumour cells from 63 patients: nine with CIN III, ten with invasive cervical cancer stage IBI-II, 20 with IIA-B and 24 with IIIB-IVB. A down regulation of HLA-I expression was observed in 84,7% of patients. 39/59 (66.1%) displayed total loss of HLA-I expression, while 11/59 (18.6%) showed partial expression. HLA-G and IL-10 up regulation was observed in 16/58 (27,6%) and 27/58 (46,6%) of cases, respectively. Most of the HLA-G positive cases show concurrent up regulation of IL-10 cytokine 14/16 (87.5%) (Spearman's rho= 0.507, P=0.000). We found also a significant association between IL-10 expression and down regulation of HLA-I (P=0.028). Among patients that over expressed IL-10 cytokine, 13/27 (48,1%) had total loss of HLA-I expression, and 7/27 (25,9%) showed down regulation. Finally, we observed a higher HLA-G expression in patients with down regulation of HLA-I expression than those with normal HLA-I expression (X2 de Pearson, P=0.004). No significant association was observed between expression of these proteins and different stages of cancer. Our results suggest that, in cervical cancer, the IL-10 expression could correlate cooperatively with up regulation of HLA-G expression and down regulation of HLA-I expression.

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P-152: Comparison of Normalization Methods for Measuring Immune Markers in Cervical Secretion Specimens

Morgan Marks[1], Rafael Irizarry[1], Yolanda Eby[1], Philip E. Castle[2], Justin Lessler[1], Patti E. Gravitt[1]

Ophthalmic sponges are used to collect undiluted cervical secretions for assessment of markers of genital tract immunity. Heterogeneity in absorbed and extracted sample volumes requires normalization in order to make valid inter-individual comparisons in marker levels. Issues with standard normalization procedures based on dilution factor calculation using sponge weight to estimate specimen volume prompted evaluation of alternative methods. We evaluated the validity of dilution factor adjustment across a range of secretion volumes as well as two alternative volume-independent methods for normalization. Sponges were collected from seventy eight (n=78) women enrolled in a study of the natural history of HPV in Perimenopause (HIP). Separately, we experimentally absorbed 5-200ul of PBS containing BSA onto sponges. Secretion volume was estimated by subtracting the weight of a representative dry sponge from a sponge after specimen collection. Total protein was extracted from each sponge in 300ul of PBS and quantified using a BCA assay. Cytokines measured using the Bio-Plex Human Cytokine 27-Plex Panel were normalized to 1) total protein; 2) dilution factor or 3) median cytokine concentration. The relationship of normalization factors and cytokine concentration pre- vs. post-normalization to specimen volume was assessed using Spearman rank correlation. Total protein and median cytokine concentration were positively correlated to specimen

volume. Dilution factor was poorly correlated to specimen volume due to inaccuracies in measuring specimen weight at low volumes resulting in over-inflated or negative values. Total protein and median cytokine normalized markers were significantly correlated to each other. Individual cytokines were less likely to be correlated to specimen volume after normalization by total protein or median cytokine methods compared to dilution factor. Dilution factors based on specimen volume suffer from loss of precision and validity at low specimen volume. We present alternative methods that minimize the effects of sampling heterogeneity on cytokine measurement. Further validation of these methods is ongoing.

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P-153: Simple and Convenient Methods for Purifying Pseudovirus of Human Papillomavirus Type 58

Hyoung Jin Kim[1], So Young Kim[1], Hong-Jin Kim[1], John T. Schiller[2]

Pseudovirus (PsV) has been used for human papillomavirus (HPV) study because native HPV do not efficiently replicate in the cell culture system. The Optiprep density gradient (OG) has been used for purifying the HPV PsV. In spite of advantage that OG provides recovering PsV with high infectious titer, its disadvantages such as requiring high priced equipment, 4 hours of centrifugation time and technical difficulty limit applicability of HPV PsV system. In this study, we introduced heparin chromatography (HC) and cation-exchange chromatography (CC) to develop a convenient purification system for HPV58 PsV, and the purification efficiencies between OG, HC and CC were evaluated. To simplify the column work of HC and CC, the columns were packed with 0.1 ml of resins, and samples were equilibrated with adding binding buffer instead of dialysis. These methods shorten the time for column working into 20 min. In addition, highly purified PsVs were obtained by two chromatographic methods. Almost all contaminants were removed as a flow-through in binding condition of 0.33 M NaCl and pH 7.0. The recovery ratios of infectious PsV titer by OG, HC and CC were 60 - 89%, 30 - 56% and 7 - 15%, respectively. Therefore, OG and HC showed superior recovery ratios while CC showed low recovery ratio. In the neutralizing antibody titration using PsVs purified by the three kinds of methods, the titers of mouse serum immunized with HPV58 PsV showed similar levels. These results indicate that HPV58 PsVs purified by HC and CC are useful for study of HPV type 58. Our two purification methods provide a simple and convenient system in obtaining highly purified PsV. In addition, two methods have considerable potential for scale-up to purify large amount of PsV. It is expected that our two purification methods could be applied to purifying different types of HPV PsV.

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P-154: Identification of Suitable Endogenous Control Molecules for MicroRNA Expression Analysis in Human Cervical Tissues

Yuanming Shen[1], Yang Li[1], Xing Xie[1]

Quantitative real-time RT-PCR (RT-qPCR), as a sensitive and reproducible quantization technique for detection of gene expression, is being used in microRNA (miRNA) expression research. Selection of optimal reference RNA targets for proper normalization is critical in miRNA RT-qPCR analysis, but it is still rather empirical. No optimal reference genes in miRNA RT-qPCR analysis have been identified in human cervical cancer up to date. In this study, we selected nine ubiquitous and stably expressed candidate reference RNA targets, including five (let-7a, miR-23a, miR-200c, miR-1979, and miR-26a) according to miRNA microarray analysis for 6 pairs of human normal and cancerous cervical tissues, and four (miR-103, miR-191, 5S rRNA, and U6 snRNA) used frequently in previous studies. All nine candidate genes were examined across 24 normal cervical tissues and 24 cervical carcinoma tissues to determine the most appropriate normalizer(s). The geNorm and NormFinder were employed for identifying the stability of gene expression and suitability for normalizors. Our results showed that all studied RNA targets reached a high expression stability with low M values(<1.5), five to nine Candidate miRNA normalizers [U6 snRNA (P = 0.39), 5S rRNA (P = 0.9), miR-23a(P = 0.5), miR-103(P = 0.12), and miR-191(P = 0.1)] were equivalently expressed between the normal and tumor groups, while the expressions of the other four Candidate miRNA normalizers (miR-1979, let7a, miR-200C, and miR-26a) were significantly increased in malignant samples (all P < 0.001). MiR-23a was identified as the most stable and reliable reference gene followed by miR-191 and miR-103. Our results suggested that miR-23a ,miR-191 and miR-103 are the optimal reference miRNAs that can be used for normalization in gene profiling studies of cervical cancer; of them, miR-23a is a novel miRNA normalizer.

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P-155: The Feasibility of PapilloCheck HPV Testing on Stored SurePath Vials

Helle Pedersen, Jalil Hariri[1]

Objectives: The effect of storage on SurePath™ specimens as regard to DNA extraction is not clearly illustrated in the literature, although some companies recommend DNA extraction within a week of sample collection. The aim of this study is to test the effect of long time storage on DNA extraction from SurePath™ specimens. **Methods:** The study included an old group of 98 leftover SurePath™ vials stored at room temperature for 2-8 weeks, and a new group of 97 leftover SurePath™ vials from the daily routine. DNA extraction was performed manually on all specimens using the PapilloCheck™ DNA extraction kit, and the SensoQuest Labcycler™ for PCR. HPV genotyping was then made using PapilloCheck™ DNA-chip and CheckScanner. **Results:** ASCUS+ was reported in 62 and 65 cases of the old and new groups respectively. The remaining specimens were WNL. Of these 40 (65%) and 46 (71%) samples harboured HR-HPV. The incidence of HR-HPV in the normal cases was on the

other hand 7/36 (19%) and 15/32 (47%) in the old and the new groups respectively. The storage time of normal and ASCUS+ specimens in the old group was identical. The age range was comparable in normal and ASCUS+ cases in both groups. **Conclusions:** It seems that 2-8 week of storage has no negative effect on HR-HPV testing in ASCUS+ cases. The significant difference of HR-HPV incidence in the normal cases is hardly due to failure in DNA extraction, because otherwise it would also have affected the ASCUS+ cases. All of the normal 36 cases in the old group, and 11 of the 32 normal cases in the new group were reported by cytotechnologists. The remaining 21 normal cases of the new group were reported by pathologists, which may explain the significant difference.

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P-156: DNA Extraction from Residual Cell-Pellet and SurePath Vial for PapilloCheck DNA-Testing

Helle Pedersen[1], Gitte Sondergaard[1], Jalil Hariri[1]

Objectives: The PapilloCheck™ DNA testing is not validated for SurePath™ residual cell-pellet. The objective of this study was to compare the DNA extraction from SurePath™ vial and the corresponding cell-pellet for PapilloCheck™ HPV DNA-testing. **Methods:** The study included 37 SurePath™ specimens reported as WNL and 62 specimens of ASCUS+. The DNA extraction from the cell-pellet as well as the vial from each case was performed manually using the PapilloCheck™ DNA extraction kit. The PCR was performed on SensoQuest Labcycler™. The PCR product was then hybridized to the PapilloCheck™ DNA-chip and scanned by CheckScanner™. **Results:** Three cases were HR-HPV positive in vials and negative in cell-pellet, while one was HR-HPV positive in cell-pellet and negative in vial. Another case was negative in vial and positive for LR-HPV in cell-pellet. HR-HPV was detected in 3 cases in the cell-pellet group, but the corresponding vials contained only LR-HPV. Otherwise there was full agreement regarding HR-HPV. One of the 3 missed cases of the cell-pellet group was reported as HSIL, and the other two were reported as WNL. All of these 3 cases harboured HR-HPV in the corresponding vials. One of the 2 missed cases in the vial group was WNL and harboured HR-HPV in the cell-pellet, while the other was reported ASCUS but harboured LR-HPV in the cell-pellet. HSIL was reported in 10 cases. One of these was HR-HPV negative in cell-pellet as well as the vial, and 1 was HR-HPV negative in cell-pellet only. When both methods were considered, no HR-HPV was missed. **Conclusion:** The study showed that the cell-pellet is feasible for DNA-testing by PapilloCheck,™ and there was no significant difference between the vial and cell-pellet groups regarding HR-HPV detecting. It also seems that the combination of both methods can be used in equivocal cases.

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P-157: Comparing PapilloCheck HPV-DNA Testing and Immunocytochemistry on SurePath Specimens

Jalil Hariri[1], Helle Pedersen[1]

Introduction: The comparison of PapilloCheckTM HPV testing (PC-HPV) and immunocytochemistry (ICC) using HPV surrogate markers is to the best of our knowledge not sufficiently studied. The aim of this pilot study was to compare the reliability of PC-HPV and ICC to detect or predict HSIL. **Methods:** PC-HPV and ICC using p16, ProExCTM, and Ki67 were performed on 37 normal and 62 ASCUS+ SurePathTM specimens. DNA extraction for PC-HPV was performed on the residual cell-pellet as well as left over material in the vials. The ICC was processed on BenchMark® XT Ventana/Roche. The ICC was interpreted positive when at least 2 of the markers were positive. Histology was available in 30 of the ASCUS+ cases. **Results:** HSIL, LSIL, ASCUS, and ASC-H were found in 10 (16%), 14 (23%), 29 (47%) and 5 (8%) out of the 62 positive smears respectively. AIS and AGC were found in 1 and 2 cases respectively. HR-HPV was negative in 2 cases of the cell-pellet group; one of these was also negative in the vial group. Otherwise HR-HPV was detected in all of the HSIL cases in cytology as well as in histology. The ICC was negative in 2 histologically verified cases of HSIL. One of these was ASCUS in cytology while the other showed HSIL, which also was HR-HPV negative in the cell-pellet group. **Conclusion:** The sensitivity of PC-HPV using vials, PC-HPV using cell-pellets and ICC to detect or predict HSIL was 91%, 82%, and 82% respectively. All 3 methods were thus comparable, although the sensitivity of PC-HPV using vials was slightly superior, but not significantly better. When all methods were used the sensitivity to detect HSIL was raised to 100%, but more efforts are needed to clarify this issue.

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P-158: Validation of L-Shape Endo/Esocervical Flocked Swab and CyMol Transport Medium with NucliSENS EasyQ® HPV v1.1 Assay

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Objectives: NucliSENS sEasyQ® HPV v1.1 (bioMérieux) is a qualitative nucleic acid assay for the detection of E6/E7 HPV oncogene mRNA (HPV16, 18, 31, 33 and 45). The L-shape endo/esocervical flocked swab (LEC) is a device for Pap collection and CyMol is a cell and nucleic acid preservation medium (Copan Italia).

Study objectives were:

1) validate the performance of LEC and CyMol medium compared to the Cervex brush (CB) (Rovers) and PreservCyt solution (Hologic) for collection and preservation of specimens for HPV detection with the NucliSENS EasyQ® HPV 1.1 assay.

2) Evaluate stability of specimens in CyMol medium after 3 weeks at room temperature (RT) and 6 weeks at -20°C. **Methods:** Six hundred clinical specimens were collected from voluntary women during a routine visit for Pap testing at 5 Fleming Lab sites in Brescia. Two clinical specimens (300 with CB in PreservCyt and 300 with LEC in CyMol) were collected from each patient in a

randomized order and classified for PAP cytology status. Aliquots of all positive specimens in CyMol medium and the same number of negative specimens were used for 3 weeks RT and 6 weeks -20°C stability study. All specimens were extracted and tested with the NucliSENS® easyMAG® and EasyQ® HPV v1.1 assay. **Results:** The results of the comparison study show a concordance of 100% between the specimens collected in CyMol and specimens collected in PreservCyt (**Table 1**). The invalid rates are comparable in the two transport media. Stability of specimens in CyMol stored at RT was demonstrated up to 3 weeks. The stability study after 6 weeks at -20°C is still in progress. **Conclusions:** No statistical difference was found between the specimens collected with cervex brush in PreservCyt solution and specimens collected with L-shape endo/esocervical flocked swab in CyMol medium when tested with NucliSENS EasyQ® HPV v1.1.

Table n°1: Concordance of the NucliSENS EasyQ® HPV v1.1 assay for the two media

		PreservCyt		
		Positive	Negative	Total
CyMol	Positive	26	0	26
	Negative	0	269	269
	Total	26	269	295

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P-159: Identification of Suitable Endogenous Control Genes for MicroRNA Gene Expression Analysis in Human Cervical Tissues

Shen Yuanming[1], Li Yang[1]

Quantitative real-time RT-PCR (RT-qPCR) is a sensitive and reproducible gene expression quantization technique which is now being used in microRNA (miRNA) expression research. As with mRNA analysis, selection of optimal reference RNA targets for proper normalization is a critical aspect of miRNA RT-qPCR data analysis. Unfortunately, the selection of reference RNA targets to normalize miRNA levels is still rather empirical and no suitable reference genes have been identified in human cervical cancer to date. In this study, first we filter five ubiquitous and stably expressed candidate reference RNA targets(let-7a, miR-23a, miR-200c, miR-1979,miR-26a) in 6 pairs of normal and malignant human cervical tissues using miRNA microarray Then the expression of these five miRNA genes and miR-103, miR-191 as well as two small nucleolar RNA genes (5S rRNA and U6 snRNA) that were already used in various studies, were examined across 24 normal cervical tissues and 24 cervical carcinoma tissues to determine the most appropriate normalizers .The expression stability and suitability of the 9 candidate reference RNA targets were validated employing geNorm and NormFinder. MiRNA23a and miRNA191 were demonstrated as the most stable reference genes and thus could be used as reference genes for normalization in gene profiling studies of cervical cancer, while the combination of two miRNAs (miRNA23a and mirRNA191) should be recommended as a much more reliable normalization strategy. This is the first study to identify reliable for analysis of miRNA by RQ-PCR in human cervical tissue.

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P-161: Confirmation of HC2 Positive/Cytology Normal Women by Papillocheck and Roche Line Blot Assay

Andrew Bailey[1], Alexandra Sargent[1], Joanna Nagorko[1], Claire Gilham[3], Henry Kitchener on behalf of the ARTISTIC Trial Study Group[2]

Introduction: With the possible addition of high risk HPV (HR HPV) testing to cervical cancer screening programmes women who present with normal cytology but who are found to be HR HPV positive pose a particular dilemma. Confirmation of the presence of HR HPV in such samples could help in the clinical management of these women. **Objectives:** To confirm the presence of high risk HPV (HR HPV) genotypes in cervical samples taken from cytology normal women who were found to be HR HPV positive using the Qiagen Hybrid Capture 2 (HC2) assay. **Methods:** Stored archival samples of cervical cells obtained from approximately 2,500 women enrolled into the ARTISTIC trial are being examined during the course of this study. All women had normal cytology but were found to contain a HR HPV type by Qiagen HC2. Nucleic acid was extracted using either the Roche MagNAPure or the Biomerieux automated extraction systems. An aliquot of DNA was then amplified and genotyped using both the Roche Prototype Line Blot (LBA) and the Greiner-Bio-One HPV genotyping assays. **Results:** Initial results obtained from testing 1281 HC2 positive/ cytology normal samples showed that 57% contained a HR HPV genotype by Papillocheck compared to 63.8% which contained a HR HPV genotype when using LBA. The ability of either assay to confirm the presence of HR HPV genotypes was highly dependent on the RLU/cut off value of the HC2 assay. **Conclusions:** The Roche prototype Line Blot assay appeared slightly more sensitive for the confirmation of HR HPV infections. These results indicate that approximately a third of women with normal cytology but who were found to be positive by HC2 positive do not have a HR HPV infection and, thus, could be managed more conservatively.

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P-165: Mapping Virus Integration Sites in HPV16+ Oral Cancers by Real Time PCR

William Seaman[1], Marion Couch[1], Jennifer Webster-Cyriaque[1]

Integration of the oncogenic HPV genome into the host chromosome is a critical event in tumor initiation. Breakpoints in the viral genome occur in the E2 gene, resulting in disruption of E2 expression and dysregulation of the E6/ E7 viral oncogenes. Early detection of integration related E2 disruption is paramount in the treatment/management of oncogenic HPV-related lesions. We have

developed a single tube multiplex assay capable of simultaneously detecting the HPV16 L1 gene as well as 3 different regions along the entire length of E2. This assay was used to determine the E2/L1 ratio in CaSki and SiHA cells. For CaSki DNA the E2:L1 copy number ratio for each E2 primer pair/probe was approximately one indicating that there is no detectable E2 disruption in these cells. Furthermore, our assay was capable of identifying the previously reported 3' E2 breakpoint in SiHA cells. We screened 65 oral cancers for the presence of HPV16 using qPCR and identified 10 oral cancers that were HPV16+. In one of these cancers, E2:L1 ratios of 1 were obtained using primer pair/probe combinations corresponding to the 5' end of E2 but no 3' E2 signal could be detected indicating that the E2 breakpoint occurred in the 3' end of the E2 gene and suggesting that there was complete integration of HPV16 genome. A low E2:L1 ratio (<0.75) was detected in 4 other oral cancer DNA samples suggesting that in these cancers there was a mixture of episomal and integrated HPV16 genomes. High E2/L1 ratios (>0.75) were detected in the other 5 cancers suggesting that high levels of HPV16 episomes were present possibly masking detection of integration events. This novel assay should allow for the detection and mapping of E2 disruption and allow for rapid screening of clinical samples for HPV16 integration events.

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P-167: APTIMA HPV Assay - Clinical Performance

Luis Martins[1], Daniela Cochicho[1], Mario Cunha[1], Carmo Ornelas[1]

The detection of HPV DNA in cervical specimens is usually made by PCR in the L1 region of the virus. The existing tests detect the presence of HPV, which is present in both transient infections, that resolve spontaneously, and in persistent infections. APTIMA HPV Assay (AHPV) detects E6/E7 mRNA of high-risk HPV types (14), using Transcription Mediated Amplification (TMA) technology. Detection of HPV mRNA may be more specific for detection of persistent infection which is more likely to progress to cervical dysplasia and cervical cancer. **Aim:** Evaluation of the AHPV sensitivity and specificity, considering the endpoint for disease the detection of >=CIN1 and >=CIN2+. **Materials and Methods:** To evaluate the clinical performance of AHPV assay, a total of 179 smears specimens - 15 negative, 25 ASCUS, 51 CIN1, 59 CIN2+ and 29 carcinomas - were analyzed by AHPV. **Results:** The % of positive samples for ASCUS, CIN1, CIN2+ and Carcinoma were 50%, 67%, 91% and 96%, respectively. Considering the >=CIN2+ as the endpoint of disease, AHPV showed a clinical sensitivity and specificity of 84% and 51%. If we lower the endpoint of disease to >=CIN1, the sensitivity increases to 89.9% and the specificity lowers to 45.9%. These results are very similar to those published: 90.8% vs 84% and 56.2% vs 51%. The Positive and Negative Predictive Value (PPV and NPV) of AHPV is 62.7% and 77.01%, respectively, for >=CIN2+. If we consider the endpoint of disease as >=CIN1, the PPN becomes 76.3% and the NPV 70%. The PPV determined has higher value than those published (32.3%) and the NPV has a lower value. **Conclusions:** This assay has a good clinical sensitivity and higher clinical specificity relative to the majority of the DNA tests. It's also a good marker of the viral activity of E6/E7 oncogenes, that can be associated with the progression to disease.

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P-168: Clinical Performance of Abbott RealTime High Risk HPV, APTIMA HPV and Papillocheck for Detection of >CIN2+ Lesions

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Background: Published studies indicate that HPV DNA testing for cervical intraepithelial neoplasia of grade 2 or 3 has a sensitivity of 94.6%, versus the 55.4% for the Pap testing. Regarding the specificity, the HPV DNA tests and Pap testing have, respectively, 94.1% and 96.8%. Generally, HPV DNA test have good sensitivity but poor specificity. Detection of HPV mRNA may improve specificity. Abbott Real Time High Risk HPV can detect 14 high risk HPV types, genotyping both HPV16 and HPV18; APTIMA HPV assay detects the oncogene E6/E7 viral activity from the same 14 high risk types and Papillocheck can detect 14+4 high risk types and 6 low risk types. **Objectives:** To evaluate clinical Specificity, Sensitivity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for >CIN2+ of the Abbott RealTime High Risk HPV (AbHPV), APTIMA HPV (AHPV) and Papillocheck (PC). **Methods:** To evaluate clinical performance of AHPV, AbHPV and PC assays for the detection of CIN2+ lesions a total of 94 smears specimens - 12 negative, 24 ASCUS, 21 CIN1, 20 CIN2+ and 17 carcinomas - were analyzed by these tests. **Results:** Overall agreement of AHPV, AbHPV and PC was 91.5% (86/94). Considering the endpoint as CIN2+, clinical sensitivity and specificity of AHPV was 97.2% and 50.9%, for AbHPV was 94.6% and 52.6% and for PC assay was 97.3% and 33.8%. All assays have high NPVs: 96.7%, 93.8% and 96%, respectively. The PC assay has a lower PPV than AHPV and AbHPV. **Conclusions:** The clinical sensitivity for CIN2+ lesions is similar for all the tests (94.6-97.3%). Relatively to specificity, the AbHPV and AHPV have similar values and PC has the lower value. These values are in agreement by those published by other authors. All tests have a high sensitivity for detection CIN2+ lesions and can be used to redefine the screening protocols for cervical cancer.

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P-169: Novel Real Time Genotyping Assay for HPV 16 E6-350G Variant Detection

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Background: it has been suggested that some Human papillomavirus (HPV) 16 E6 variants (for instance T350G) could be involved in viral persistence and progression of HPV infection. Detection of

these variants by DNA sequencing is expensive and time consuming.
Objective: To evaluate a novel RT- PCR (Real Time PCR) assay for detection of HPV 16 350G Variant.
Materials and Methods: For identification of HPV 16 sequence variation, 761bp from the LCR-E6 region (Nt 7701-591) were amplified (Expand HiFi, Roche) as previously described (Xi LF, 2006), sequenced with the same primers (BigDye 1.1. Abi Prism 3100-Avant), and compared with NCBI database. For the T350G discrimination assay two different labeled probes were used: wild strain was detected in the VIC-filter and mutant strain in the FAM-filter (GTXpress Master Mix. 7500 Fast. Applied Biosystems). Primers and Taqman-MGB probes were designed targeting the conserved region between the different HPV 16 subtypes. Analytical performance was analyzed testing of 10-fold serial dilutions of the HPV 16 WHO International Standard (NIBSC 06/202) and DNA of HPV 16 LCR-E6 region from a wild strain, a 350G mutant strain, and mixes of both (350K). Clinical sensitivity and specificity were calculated by performing both RT-PCR and LCR-E6 sequencing in 121 endocervical samples positive for HPV16 in routine screening.
Results: For the T350G discrimination RT-PCR assay, the cut-off value was aprox $5.0x10^3$ vp/ml for T350T, for T350G and for T350G detection in mixtures containing 20% of this mutant. The results of both RT-PCR and DNA sequencing were, respectively: 65/65 350G variant, 4/4 350K variant, 52/52 350T. In comparison with DNA sequencing, the novel assay sensitivity and specificity were 100% for the 350G Variant detection.
Conclusion: This novel real time genotyping assay is a rapid, sensitive and specific method for detection of HPV 16 E6-350G Variant.

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P-170: Lack of Type-Specific Concordance of Roche Linear Array® and Innogenetics InnoLiPa® Performed on Different DNA Extracts

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Background: A plethora of manual and automated DNA extraction systems are used for the specimen preparation prior to HPV testing, not always in compliance with the guidelines of kit manufacturers.
Objectives: To investigate the influence of different DNA extraction methods on HPV typing by Linear Array® (LA, Roche) and InnoLiPa® (IL, Innogenetics).
Methods: Twenty samples, which tested positive by LA using the Roche-recommended AmpliLute® extraction, were selected. Two other methods were used for DNA extraction from the original PreservCyt® cervical samples: the QiAmp® Blood mini kit (Qiagen) and the NucliSens EasyMAG® automated system (bioMérieux). LA and IL were performed in parallel on the three different extracts.
Results: Type-specific concordance (i.e. same HPV types found in all the three extracts) was observed in 11/20 samples (55%) for LA and 15/20 (75%) for IL. When comparing the results obtained on the DNA extracted with the two manual methods, 5/20 (25%) and 4/20 (20%) cases were not concordant by LA and IL, respectively. When comparing LA results obtained on any of the manual extracts with those obtained after automated extraction, 7/20 samples (35%) did not show type-specific concordance in both cases. IL results were non concordant in 4/20 cases (20%) when comparing the AmpliLute® vs. the automated DNA extraction, while non concordant results were observed in 3/20 cases (15%) when comparing the Qiagen vs. the automated extraction. Lack of type-specific reproducibility in LA concerned 9 HPVs (2 LR and 7 possible HR or HR), mostly with medium/high inclusivity level (i.e. types the LA detects at higher concentration level).
Conclusions: HPV genotyping test performance is significantly affected by the DNA extraction method, although at lesser extent for IL. Use of consistent protocols for sample preparation is a priority to guarantee intra-assay and intra-laboratory reproducibility over time, particularly when monitoring transient vs. persistent HPV infections.

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P-171: Universal HPV Genotyping of GP5+/6+-, Amplicor- and PGMY-Amplimers by Digene RH and LQ Tests

D.T. Geraets [1], L.J. van Doorn [1], W.G.V. Quint [1], W.J.G. Melchers [2]

Background: The Amplicor HPV test (Amplicor, Roche) permits the simultaneous detection of 13 HR-HPV types in a single reaction. However, to identify the specific HPV genotype, a separate PGMY-based amplification is required to perform the Linear Array HPV Genotyping Test (LA, Roche).
Objectives: The goal of the current study was to test whether the novel digene HPV Genotyping RH (reverse hybridization-based) and LQ Tests (xMAP-based) (QIAGEN, GP5+/6+ PCR-based identification of 18 classified HR-HPV genotypes) allow direct genotyping of Amplicor- and PGMY-based amplimers.
Methods: HPV-positive cervical scrapes for the current study were pre-selected based on genotyping results by a different method. A selection of these HPV-positive cervical samples was amplified and analyzed by Amplicor (n=258), while another panel of cervical specimens was PGMY-amplified and genotyped by LA (n=66). Both Amplicor- and PGMY-amplimers were additionally genotyped by the digene RH Test and LQ Tests.
Results: Amplicor-amplimers (n=258): 193/258 samples were Amplicor-positive, and these amplimers could be directly genotyped for 13 HR types by RH Test (183/193) and LQ Test (189/193). 65/258 samples remained Amplicor-negative, but contained one of 13 HR-HPV genotypes in 6/65 by RH Test and in 8/65 by the LQ Test. In addition, genotyping revealed untargeted types (HPV66 and 82) that were amplified but undetected by Amplicor.

PGMY-amplimers (n=66): Of 66 samples genotyped for presence of 18 HR types, 26 were positive by LA, 25 by RH Test, and 26 by LQ Test. RH and LQ Test demonstrated good agreement with LA for HPV genotyping.
Conclusions: The digene HPV Genotyping RH and LQ Tests can be directly applied on amplimers generated by the GP5+/6+, Amplicor, and PGMY systems. The use of a universal genotyping test could contribute to standardization of HPV testing for clinical practice.

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P-173: Analytical Validation of the Digene HPV Genotyping RH and LQ Tests

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Background: The GP5+/6+-based digene HPV Genotyping RH and LQ Tests were recently introduced for identification of 18 HPV genotypes and 2 subtypes (i.e., HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 (68a), 73, 82MM4 (82IS39)) in HC2-positive samples. Analytical validation data are presented here.
Methods: Assays were validated according to ICH guidelines for, among others, analytical sensitivity, analytical specificity, reproducibility, accuracy and robustness.
Results: Analytical sensitivity Formal Limit of Detection (LOD) for HPV16, 18, and 45 ranged between 4 and 23 copies/PCR. Analytical sensitivities for the remaining 15 types varied between 1 and 100,000 copies/PCR.
Analytical specificity: The assays did not cross-react with non-targeted types. In addition, no other pathogens commonly found in the female anogenital tract were reactive.
Reproducibility: Plasmids representing all targeted HPV types and 92 clinical samples were assayed in replicates on different days by different technicians with different kit lots. The results showed 100% identical genotypes in the plasmid panel and more than 95% reproducibility for clinical samples.
Accuracy: One hundred and eight clinical samples were tested in parallel by digene RH and LQ Tests and the homebrew GP5+/6+-PCR RLB system. Initial agreement was 91% and 93% with the RH and LQ Test, respectively. After discordant resolution the agreement was 98% for both assays.
Robustness: The assay results remained unaffected by deliberate variations in relevant method parameters, such as increased or decreased PCR temperature conditions, hybridization temperatures, and incubation times.
Interfering substances: The presence of blood and other potentially interfering substances had no effect on the assays.
Conclusions: The digene RH and LQ Tests show good analytical performance characteristics. Performance evaluation on clinical samples is ongoing. However, it is important to realize that assay efficacy is determined by the entire diagnostic algorithm comprising DNA isolation, PCR and amplimer analysis.

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P-174: Evaluation of Test Performance Characteristics and Operational Parameters of 5 HPV Genotyping Assays

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Background: Identification of HPV types is required for epidemiologic measurements of vaccine efficacy and type-specific risk for cervical cancer. In order to identify the most suitable genotyping platform for population studies, we evaluated 5 genotyping platforms against a panel of 355 liquid based cytological (LBC) samples.
Methods: The testing panel of 355 samples consisted of N=171 cervical LBC from women who were referred to colposcopy for follow up and N=184 intra-anal LBC from HIV-positive men with clinical history of abnormal anal pathology. Nucleic acid was extracted from LBC by the AmpLilute kit (Roche), amplified as per individual assay protocol and detected with the Roche Linear Array (LA), INFINITI HPV 26-plex (INF), Seeplex HPV18 ASE (SG), Qiagen 16-plex (QIA), and an in-house Luminex (LU) assay. LA detection of HPV52 was confirmed by PCR.
Results: In order to overcome the lack of a "gold standard" when assessing test performance, we defined a HPV consensus result when 3 tests detected the same HPV type. Since only the 13 HR HPV types are commonly represented in all 5 tests, detailed comparative analysis was focused on HR HPV. The analytical sensitivity/specificity for HR HPV detection ranged from 10% to 100% for individual HPV types. Pair-wise test comparisons revealed that concordance varied by HPV type and kappa values ranged from 0.052 to 0.862 (poor to excellent). The tests also varied in their ability to detect co-infection with multiple HPV types. Infection with >3 HPV types was detected in 31.9%, 26.5% and 31.5% of samples by the LU, SG and LA assays and in 11% by the INF and QIA assays. Cost, throughput and labour varied between assays.
Conclusions: HPV genotyping assays vary in test performance and operational parameters. Our data will inform the decision framework for the laboratory component of HPV surveillance and vaccine evaluation.

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P-175: Reproducibility of Early Morning Oral Rinse Self Sampling for Detection of Oral HPV

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Background: Oral HPV has been implicated in the etiology of malignant oral disease. However, reported rates of oral HPV vary by methodologies to collect specimens and the

sensitivity of assays used. We hypothesized that a specimen obtained upon arising in the morning would provide more exfoliated cells and a better substrate for HPV DNA detection. **Methods:** We selected an HIV positive population attending an inner city oral health clinic with high expected prevalence of oral HPV infection. Oral rinse specimens were obtained from 52 consenting adults at two time points within the same day: samples were collected upon arising in the morning and approximately 8 hours later in clinic and tested for HPV DNA by MY09/11 and FAP PCR. Kappa statistics were used to assess agreement between paired samples. **Results:** The detection rate of HPV DNA in oral rinse samples ranged from 53.8% for samples collected in the afternoon to 59.6% for morning samples. Sixteen (30.8%) individuals tested positive for high-risk HPV and 34 (65.4%) for low-risk types. There was good agreement in detection of HPV DNA between morning and afternoon samples (kappa=0.57, 95%CI: 0.35-0.79). This increased when restricting for high-risk types detected in either morning (28.9%) or afternoon (23.1%, kappa=0.75, 95%CI: 0.55-0.96). Distribution in number of concurrent types was also similar for morning and afternoon samples; 17 (54.8%) and 11 (39.3%) samples, respectively were positive for a single type, whereas 11 (35.5%) and 12 (42.9%) had multiple HPV. **Conclusion:** Although HPV was detected more often in early morning oral rinse samples, detection rates on the same day (at home and in clinic) were similar. Self collected oral rinse sampling may provide a potential method for monitoring oral HPV infection.

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P-176: Evaluation of Dry and Wet Transported Cervical Exfoliated Samples for Detection of Human Papillomavirus Infection

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We determined the feasibility of HPV detection in cervical exfoliated cells collected as dry swab samples. Both dry cervical swab and STM cervical swab samples were collected from 135 patients attending either colposcopy or women's clinics in Guayaquil, Ecuador, who had a cytology diagnosis within 6 months. HPV was detected by dot blot hybridization and genotyped by the liquid bead microarray (LBMA) assay. Overall, 23.1% of dry samples were positive for any high-risk HPV types, and 24.6% of STM samples were positive for any high-risk HPV types. Of 125 paired samples, the type-specific high-risk HPV proportion positive agreement was 60.7% (kappa 0.69, 95% CI: 0.53-0.82). We found that dry samples were more likely to be insufficient for HPV testing than STM samples. Consistent with this observation, we showed that genomic DNA quantitated by β-actin gene was almost 20 times lower in dry samples than in STM samples when detected by the real time Taqman assay, while HPV DNA viral loads in dry samples were only 1.6 times lower than in matched STM samples. We concluded that cervical exfoliated cells could be collected as dry swab samples for HPV detection.

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P-177: Enrichment of HPV-Integrated gDNA from FFPE Tissue for Next-Generation Sequencing

Matthew A. Titmus[1], Eli Hatchwell[1], Kenneth R. Shroyer[1]

Integration of the human papillomavirus episome into the host cellular genome is a key step in the progression from HPV infection to oncogenesis. In HPV-induced cancers this event has been correlated with constitutive expression of the HPV E6 and E7 oncoproteins. These oncoproteins negatively interfere with host p53 and pRb, respectively, resulting in an accelerated cell cycle and increased host genomic instability. It is widely assumed that this E6/E7 over-expression is due in the majority of cases to the simultaneous disruption of the E2 ORF, whose product acts to restrict E6/E7 expression in intact HPV. The process of integration occurs in an undirected fashion and doesn't appear to follow any consistent pattern with regard to integration sites in either the host or viral DNA. This fact, and the observation that integrated HPV DNA appears to have an increased rate of mutation, limits the usefulness of PCR-based approaches for analysis of integration events. As a result, what little is currently known about HPV integration has been stitched together from a variety of studies employing a variety of experimental approaches which, by necessity, have generally been both low-resolution and reliant on a small number of samples. The development of high-throughput sequencing has provided us with the means to overcome these long-standing limitations by not only making it possible to simultaneously produce thousands or millions of sequences, but to do so without a detailed knowledge of the target's flanking regions. To take advantage of this technology, our lab has developed a hybridization-based method to enriching genomic DNA recovered from archival formalin-fixed paraffin-embedded (FFPE) tissues for HPV-integrated sequences to a degree suitable for massively parallel sequencing. Using this approach, it is possible to sequence the regions surrounding the host:virus interfaces of a large number of HPV-integrated samples simultaneously.

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P-179: Prevalence of Human Papillomavirus Types and Viral Load - Pap Test Correlation in Romania

Ramona Gabriela Ursu[1], Luminita Smaranda Iancu[1]

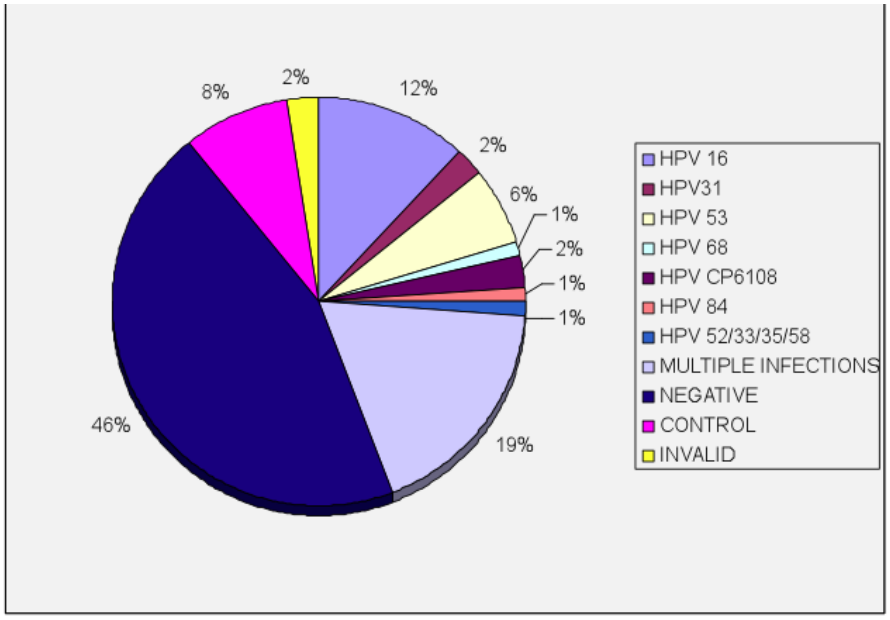
Objectives: To estimate the HPV genotype prevalence in North East Romania, and to check the utility of HPV 16 viral load as a

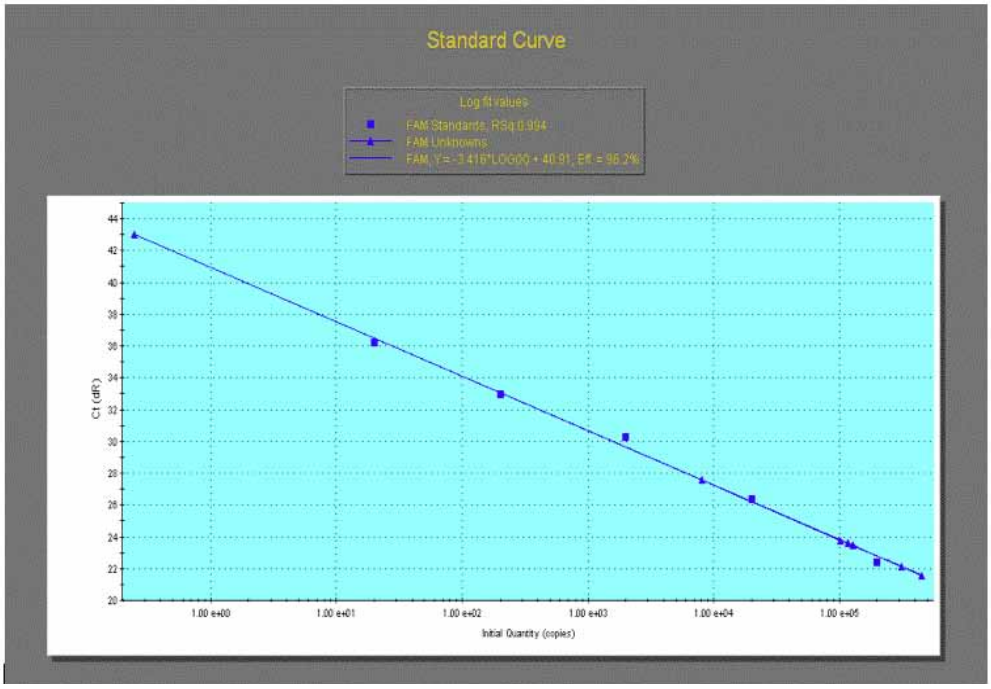
biomarker, for detecting women in early stages of HPV infection, before developing cervical cancer.

Methods: We tested cervical cell specimens collected from 84 women who signed the informed consent approved by the local Bioethical Committee. The age range of women tested for HPV was from 26 to 57 years, and the Pap test was performed according with Bethesda classification. HPV genotyping was performed with Linear Array HPV Genotyping (ROCHE DIAGNOSTICS®) and PCR reaction was realized with ABI 9700 Gold Plate System. The viral load of HPV 16 positive samples was quantified with Path-HPV16 Real-time PCR detection kit for Human Papillomavirus, MX3000P STRATAGENE instrument.

Results: Among the tested samples, 38 (46%) were negative for any HPV type, 15 (19%) patients presented multiple infections with oncogenic and non-oncogenic HPV types (16, 18, 26, 33, 35, 39, 42, 51, 54, 58, 52/33/35/58, 59, 61, CP6108, 62, 81), 10 (12%) were positive only with HPV 16, 5 (6%) for HPV 53, 2 (2%) were positive for HPV 31, 1 (1%) for HPV 52/33/35/58, 1 (1%) for HPV 68 and one (1%) for HPV 84 (fig. 1). Parameters of standard curve (efficiency, pipetting, reproducibility, slope) were between the accepted limits: Eff = 96,2%, slope = 3,416, Rsq = 0,994 (fig. 2) The viral load of tested HPV 16 positive samples was between 8,025e+003 - 4,549e+005 copies.

Conclusions: The epidemiological data provides information about distribution of HPV genotypes and may be important for determining the future impact of vaccines and potential changes in the country's epidemiological HPV profile. The prevalence of HPV 16 is similar to the results of others studies. High viral load of HPV 16 was associated with HSIL. We must continue our study on more cases, to have a broader image in our region.





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P-180: Mycobacterium Associated Immune Reconstitution with Highly Acrive Antiretroviral Therapy (HAART) Initiation

Nasuna Florence[1]

Issues: The high prevalence of HIV/ HPV and TB co-infection is evident in sub-Saharan Africa. As antiretroviral use increases in this setting, it is imperative that we examine the impact of treatment intitiation where HIV/HPV and TB co-infection exist.

Description: We describe the case of a 51 year-old Ugandan woman with HIV/HPV / TB co-infection and a T-cell count of 18. The patient was diagnosed with extrapulmonary TB following aspiration of a supraclavicular abscess that decreased in size after intensive phase TB therapy. She initiated HAART thereafter. Twenty-nine days after antiretroviral treatment began she experienced recrudescence of symptoms with marked inflamation, local pain, fever and a fluctuant nodule at the prior site of infection. Aspirate of this revealed 3+ acid-fast bacilli on smear without evidence for other organisms. Culture results are pending.

Lessons learned: HAART restores the immune response to mycobacterium disease.

Recommendation: In this era where access to antiretroviral therapy in Africa is becoming more widely available, clinicians should be alert to the manisfestations of immune reconstitution syndromes. Particularly in settings where HIV/HPV/TB endemicity is high and where antiretroviral therapy is often initiated in advanced stages of HIV/HPV disease, providers ought to be vigilant for the development of acute mycobacterial symptoms and prepared to institute steroid therapy as warranted.

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P-181: Colposcopy Assessment Does not Predict the Follow-up in Women with hr-hpv Infection and Mild Cytological Abnormalities

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Background: Colposcopy examination of women with ASC-US or LSIL detects CIN2-3 in 5-20%; 10-15% of the rest of them develop CIN2-3 after 2 years follow-up. These facts justify that current protocols recommend colposcopy examination as an integral part of the follow-up of these women. Nevertheless, it is unknown whether colposcopy findings can provide any valuable information that might help to predict which patients will progress.

Objective: To compare the outcomes of women positive for HR-HPV with normal cytology, LSIL or ASC according to the colposcopy findings at initial evaluation.

Patients and methods: From January 2000 to July 2008, women referred to the Colposcopy Unit of the Hospital Clinic of Barcelona due to an abnormal cytology and/or HR-HPV infection who, after an exam including cytology, colposcopy (with colposcopically directed biopsy or endocervical curettage if indicated) and HPV detection, fulfilled following inclusion criteria were prospectively recruited: 1) positive HR-HPV testing, 2) negative, ASC or LSIL cytology. Follow-up controls were scheduled every 6 months. Colposcopy findings were recorded following the International Federation for Cervical Pathology and Colposcopy (Barcelona 2002) criteria. Patients were grouped according to their follow-up outcome: regression, defined as negative Pap, and negative HR-HPV detection; persistence, established on the basis of LSIL/CIN1 diagnosis and/or persistent HR-HPV infection; progression, defined as histological diagnosis of CIN2/3.

Results and conclusion: 465 women were recruited (mean age 33.6 ± 10.4 years). Diagnoses at enrolment were: normal cytology in 92 women (19.8%), ASC in 67 (14.4%) and LSIL/CIN1 in 306 (65.8%). Table 1 shows colposcopy findings according to the initial diagnoses. No significant differences were found between groups (p=0.491). After a mean follow-up of 19.5 months (range 12.0-90.9), 161 (34.6%) women regressed, 261 (56.1%) showed a persistent lesion and 43 (9.3%) progressed. Table 2 shows the outcomes according to initial colposcopy findings (p=0.284). 21/174 (12.1%) of the women with an abnormal transformation zone (ATZ) involving <50% of the cervical surface, 2/38 (5.3%) of women with ATZ involving >50% and 1/4 (25.0%) of those showing only endocervical lesion progressed (p= 0.228). In conclusion, in women with HR-HPV infection and minimal or no cytological abnormalities neither colposcopy characteristics nor lesion surface area were related to progression risk.

Colposcopy findings at enrolment		Initial cytological result			p
		NEG	ASC-US	L-SIL	
Normal (n= 249)	n (%)	55 (22.1%)	39 (15.7%)	155 (62.2%)	0.491
Minor changes (n= 199)	n (%)	34 (17.1%)	25 (12.6%)	140 (70.4%)	
Major changes (n= 17)	n (%)	3 (17.6%)	3 (17.6%)	11 (64.7%)	

Colposcopy findings at enrolment	Outcome after follow-up			p
	Regression	Persistence	Progression	
Normal	95 (38.2%) (32.3%-44.3%)	135 (54.2%) (48.0%-60.3%)	19 (7.6%) (4.9%- 11.6%)	0.284
Minor changes	59 (29.6%) (23.7%-36.3%)	117 (58.8%) (51.9%-65.4%)	23 (11.6%) (7.8%-16.7%)	
Major changes	7 (41.2%) (21.6%-64.0%)	9 (52.9%) (31.0%-73.8%)	1 (5.9%) (1.1%-27.0%)	

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P-183: Updated Data from the Pregnancy Registry for Gardasil® (Human Papillomavirus Types 6/11/16/18 Vaccine)

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Objectives: The Pregnancy Registry for Gardasil (Merck) collects voluntary post-marketing reports of pregnancy exposures from health care providers or the public. This report covers the period from product licensure (June 2006) through May 31, 2009. **Methods:** The registry is conducted in the US, Canada and France. Enrollment criteria include an identifiable health care provider and patient, with vaccine exposure occurring within 1 month prior to the date of onset of the last menstrual period (LMP) or during pregnancy. The primary outcomes of interest are birth defects, live births, fetal deaths, elective terminations, and spontaneous abortions. Outcomes from prospectively reported pregnancy exposures with estimated dates of delivery within the report period are used to calculate rates. **Results:** 1897 women were enrolled in the registry with 1636 exposures reported prospectively (before the outcome of the pregnancy was known). Of the prospective reports with known outcome, there were 65 elective abortions, 64 spontaneous abortions, 2 ectopic pregnancies, 10 fetal deaths, and 968 live births, which included 6 sets of twins. Of 974 infants, 926 were normal infants with no defects, 21 had major and 26 had minor congenital anomalies. The overall rate of spontaneous abortion was 6.1 per 100 outcomes (95%CI: 4.7 to 8.0). The prevalence of congenital anomalies at birth was 2.4 cases per 100 live born infants (95%CI 1.5, 3.5). Of the 10 fetal deaths, 1 case included Turner's syndrome, 3 had attributable causes, and 3 had a history which might have contributed to the outcome. **Conclusions:** Rates of spontaneous abortions and major birth defects were comparable to those reported in the literature (15% and 2.67%, respectively). Although the numbers of exposures are not sufficient to rule out a low risk, data collected in the pregnancy registry to date do not support a relationship between vaccine exposure and adverse fetal outcomes.

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P-184: Defective Natural Killer Cell (NK) Function in Patients with Recurrent Respiratory Papillomatosis (RRP)

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Introduction: We recently showed an altered frequency of killer cell immunoglobulin-like receptor (KIR) gene haplotypes in RRP. Specifically, KIR frequency haplotypes lacking 3 activating KIR genes, KIR3DS1, KIR2DS1, KIR2DS5, predicted disease severity. This suggested that these KIRs may be required to remove HPV-infected keratinocytes without HLA class I expression observed in papillomas. Reduced/absent HLA class I expression should prevent inhibitory KIR molecule-ligation on NK cells, and release NK inhibition of NK cytolysis, permitting activating KIR ligation by viral epitopes in/outside of the context of HLA class I molecules. **Methods:** To determine if NK cells obtained from the blood of patients with RRP can cause cytolysis of target cells, we performed a CD107a mobilization assay using NK cells from 12 RRP patients and 13 controls. CD107a is a cell surface marker transiently expressed after cytolytic granule release, and correlates with cytokine secretion and NK cytolysis. PBMC were cultured 4hrs in media alone or with the HLA class I deficient cell line K562, at an effector:target ratio (E:T) of 5:1 in the presence of FITC-conjugated anti-CD107a and monensin. Following incubation, cells were stained with the amine-reactive live/dead fixable violet dead cell stain kit and CD3 APC, CD56 PE, CD14 and CD19 PerCP. Samples were analyzed by flow cytometry. **Results:** The percentage of CD107a expression on CD3-56+ NK cells after subtraction of the spontaneous activity showed a significant reduction of CD107a expression on NK cells in patients with RRP, vs controls (p=0.044). **Conclusion:** There is defective NK cellular innate responses

in RRP, manifest as the failure of NK cells to be activated by target cells lacking HLA class I. Thus, papillomas containing keratinocytes lacking HLA class I are not likely to be cleared by NK cells in papillomas, thus adding cellular innate immune dysregulation to adaptive TH2-like/Treg polarization present in patients with RRP.

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P-187: Future Directions for Screening: Modelling Results from the ARTISTIC Trial

Rosa Legood[1], Milena Farco[2], Chris Roberts[2], Henry Kitchener[2]

Background: Currently there are a range of future options for cervical cancer screening including primary HPV testing utilising hybrid capture II, cytology followed by HPV triage and further combinations based on type 16/18 testing. Future directions in research also need to consider the implications of strategies for the post-vaccination era. The objective of this modelling study was to utilise ARTISTIC trial data to assess the potential cost-effectiveness of alternative cervical cancer screening strategies to help identify likely optimal strategies going forward.

Methods: The setting of the ARTISTIC trial was Greater Manchester in the UK, and the study population were 24,000 women aged 20-64 who underwent primary cervical cancer screening. The trial cohort was randomised to combined screening with human papillomavirus (HPV) testing and liquid based cytology (LBC), or screening only with LBC (though HPV tests were performed and the results concealed). Genotyping data was also collected as part of the trial protocol. In this study, clinical data from the first round of the trial were re-analysed and split by age group to assess the within trial colposcopy outcomes of alternative management strategies and matched with data on initial HPV types. A mathematical model was developed incorporating both trial data and further evidence synthesis, to predict management outcomes utilising alternative potential management strategies for both an unvaccinated and vaccinated cohort. Cost data has already been collected alongside the trial and further indicative prices of novel testing strategies were then obtained from the companies. The comparative cost per case detected of alternative management strategies over one screening round were then assessed in a stochastic framework.

Results: Results from the study will be presented at the meeting on the cost-effectiveness of alternative management strategies. These data will be utilised to inform on likely potential cost-effective options for cervical cancer screening.

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P-188: Cytology and Genotyping as Secondary Screens after Primary HPV Screening

Hui Du[1], Bin Yang[2], Xinfeng Qu[3], Suzanne Belinson[4], Robert Pretorius[5], Gao Yang[6], Chun Wang[1], Jingjing Li[6], Ruifang Wu[1], Jerome L. Belinson[4]

Objectives: The Shenzhen Cervical Cancer Screening Trial II (SHENCCAST II) is a 10,000 woman study examining self-sampling, multiple HPV technologies, cytology with computer assisted detection, and genotyping. We report here an evaluation of cytology and genotyping as secondary screens after primary HPV testing.

Methods: Women from 3 sites in rural Guangdong Province and city sites in Shenzhen, China were eligible if non-pregnant, 25-59 yrs of age, no screening ≥ 3 years, no hysterectomy, and no pelvic radiation. 10,000 women will be screened with self and direct HPV testing as well as ThinPrep cytology with computer assisted detection. The entire study population will be genotyped for 14 high-risk HPV types both on their self and direct samples using the high-throughput mass spectrometry based system MALDI-TOF. Positivity by any HPV test and/or cytology led to colposcopy where all patients were colposcoped and biopsied using the POI biopsy protocol of directed and random biopsies (minimum 5/patient).

Results and Conclusions: 4965 women are included in this interim analysis (full 10,000 will be presented). Mean age 38.8 years, >=ASCUS = 549/4965 (11.06%), >=LGSIL= 185/4965 (3.73%),>=HGSIL = 98/4965 (1.97%);>=CIN II = 148/4965 (2.98%), >=CIN III = 95/4965 (1.91%); HPV + rates for direct HC-II 725/4965 (14.60%), HPV + rates for self-sampling Maldi 791/4965 (15.93%). A clearly superior secondary screen is not identified. All options have strengths and major weaknesses depending on the clinical setting.

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P-189: Primary HPV Screening with Secondary VIA Followed by Cryotherapy

David Starks[1], Lucybeth Nieves-Arriba[1], Christine Enerson[2], Suzanne Belinson[3], Christine Booth[4], Jennifer Brainard[4], Andres Chiesa-Vottero[4], Jerome L. Belinson[3]

Objective: To determine the effectiveness of triage VIA after primary HPV screening, for prevention of undertreament when performing cryotherapy prior to biopsy confirmation.

Methods: In the Mexican Cervical Cancer Screening Trial II (MECCS II) participants were women ages 30-50, non-pregnant, variable screening histories, and no prior hysterectomy or pelvic irradiation. All obtained a self-sample using the 4th generation POI/NIH brush. The sample was placed in PreservCyt for the high-risk HPV assays (HC-II and APTIMA). Then two direct cervical samples were obtained for cytology, HC-II, and APTIMA. Subjects positive on any test were recalled. At the 2nd visit, triage VIA was used to identify preinvasive disease too large for the cryo probe (>3 quadrant disease) or cancer. All patients were

then colposcoped using the POI protocol of directed and random biopsies. All HC II positive subjects eligible by VIA triage were treated with cryotherapy. All patients treated with cryotherapy were requested to return in 6 months. At 6 months, a direct sample was obtained for cytology, HC-II, and APTIMA. All subjects who had abnormal biopsies prior to cryotherapy, or whose cervix appeared abnormal, were colposcoped and re-biopsied. **Results/Conclusions:** 2512 women were included in the study with a mean age of 39 years. 300 received cryotherapy (297 with complete data). 81% received follow-up, 231 at 6 months and 10 by referral MD. 229/231 have complete 6 month data. Prior to cryotherapy 30/297 women (10%) had >=CIN 2 (15/30 ECC positive, 2/30 >3 quadrant disease). At follow up 5 women had >=CIN 2 (2/5 on ECC) and there were no cases of >= 3 quadrant disease. Aptima detected all five high grade lesions, cytology and HC-II each missed a CIN2. VIA triage does not evaluate the endocervix effectively, at 6 months 9/11(82%) with prior positive ECCs tested negative after cryotherapy.

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P-190: Prospective Randomized Comparison of the POI/NIH and the Qiagen Self-Sampling Brushes

Jerome L. Belinson[1], Hui Du[2], Bin Yang[3], Suzanne Belinson[1], Xinfeng Qu[4], Phillip Castle[5], Robert Pretorius[6], Chun Wang[2], Gao Yang[7], Ruifang Wu[2]

Objective: Compare the sensitivity and specificity for detection of ≥CIN 2 of two vaginal self-collection devices (POI/NIH brush vs. Qiagen brush) using HR-HPV assays Cervista, Maldi-TOF, and HC2. **Methods:** 10,000 women age 25 to 59 years residing in Guangdong Province, China who were recruited for the Shenzhen Cervical Cancer Screening Trial II (SHENCCAST II). Women were randomly assigned to self-collect vaginal specimens with either the POI/NIH self-sampler or the Qiagen conical-shaped brush. All subjects had cervical specimens collected with the broom sampler by doctor directly and placed in PreservCyt for cytology and HPV testing. Cervical cytology specimens were prepared by the ThinPrep method. Vaginal self-collected specimens were tested for HR-HPV by Cervista and Maldi-TOF. The direct cervical specimens were tested for HR-HPV by HC2, Cervista, and Maldi-TOF assays. Women with HR-HPV in direct cervical or vaginal self-collected specimens or with cervical cytology of ASC-US or greater underwent colposcopy with our 5-biopsy protocol. **Results:** This interim analysis includes the first 4,965 participating women. Their mean age was 38.8 years. 3% (148/4965) were diagnosed with ≥CIN 2. For direct cervical specimens HC 2 (sensitivity 94.6%), Cervista (90.5%) and MALDI-TOF (92.6%) were on similar ROC curves. Using Cervista for detection of HR-HPV, the sensitivity for ≥CIN 2 of the POI/NIH sampler (76.4%) was similar to that of the Qiagen brush (71.1%) p=0.58 . Using Maldi-TOF for detection of HR-HPV, the sensitivity for ≥CIN 2 of the POI/NIH sampler (95.8%) was similar to that of the Qiagen brush (88.2%) p=.16) and similar to that of direct cervical Maldi-TOF (92.6%) p>0.05. **Conclusion:** For direct cervical specimens, areas under the ROC curves for Maldi-TOF, Cervista, and HC2 are similar; for vaginal self-collected specimens, the sensitivity and specificity for ≥CIN 2 of the POI/NIH sampler tested with Maldi-TOF is similar to that of the direct cervical specimens.

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P-191: The Shenzhen Cervical Cancer Screening Trial II (SHENCCAST II)

Ruifang Wu[1], Hui Du[1], Suzanne Belinson[2], Bin Yang[3], Xinfeng Qu[4], Chun Wang[2], Ruosong Wu[4], Guoping Wang[1], Gao Yang[5], Jerome L. Belinson[2]

Objectives: To test a new self sampling device, 2 new HPV platforms and a new computer assisted detection system in a population based screening trial.
Methods: 10,000 women were screened in Guangdong Province, China, if non-pregnant, 25-59 yrs of age, no screening < 3 years, no hysterectomy, and no pelvic radiation. Self-sampling was block randomized between the Qiagen brush and the new POI/NIH brush and tested with Cervista and MALDI-TOF for HR-HPV. Direct cervical sampling was placed in PreservCyt, from which Cytology, HC-II, Cervista, and MALDI-TOF were processed. All women with ≥ASCUS, and/or positive self or direct HR-HPV were asked to return for colposcopy and biopsies using the POI directed and random biopsy protocol.
Results: 4965 women are included at this time in this interim analysis, Mean age 38.8 years, ≥ASCUS = 549/4965 (11.06%), ≥ HSIL = 98/4965 (1.97%); ≥ CIN II = 148/4965 (2.98%), ≥ CIN III = 95/4965 (1.91%); HPV + rates for direct tests HC-II 725/4965 (14.60%), Cervista 607/4965 (12.23%), Maldi 693/4965 (13.96%). HPV + rates for self-sampling: Cervista 901/4965 (18.15%), Maldi 791/4965 (15.93%). Cytology using the I2 imager had a sensitivity and specificity (≥ASCUS) for ≥ CIN II of 89.9% and 91.4% respectively. The areas under the ROC curves for the direct HC-II, Cervista, and the Maldi were similar p=0.088. Using Cervista the sensitivity for ≥ CIN II of the POI/NIH self-sampler was similar to the self-sampling Qiagen brush (76.4% vs.71.1% respectively, p=0.58). Using Maldi-TOF for detection of HR-HPV, the sensitivity for ≥ CIN II of POI/NIH self-sampler was similar to self-sampling Qiagen brush (95.8% vs. 88.2% respectively, p=0.16) and similar to that of direct Maldi-TOF (92.6%) p>0.05.
Conclusions: The I2 imager functions as an excellent "cytotech" surrogate. All the HPV assays worked similarly well as direct tests, and the POI/NIH self-sampler with a PCR based assay equals their performance.

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P-192: Effects of Purification Method in Antigenicity and Immunogenicity of Human Papillomavirus type 16 Virus-Like Particle from Saccharomyces cerevisiae

Su Jeung Lim[1], Hyoung Jin Kim[1], Hong-Jin Kim[1]

In the virus-like particle (VLP) of human papillomavirus (HPV), conformational epitopes are an important factor affecting induction of neutralizing antibodies in the use of prophylactic vaccine. The structure of conformational epitope can be altered by binding with ligand. However, there has been no research investigating the effects of purification method in antigenicity and immunogenicity of VLP although the interaction between VLP and ligand on column resin is a potential factor affecting the structure of conformational epitope. Recently, we developed two one-step chromatographic methods for purifying the HPV VLP from Saccharomyces cerevisiae. One method uses heparin chromatography and the other, cation-exchange chromatography. In this study, we compared assembly characteristics and immune responses in mouse model between VLPs purified by the two methods above. As a result, it was confirmed that the VLPs purified by heparin chromatography (hHPV16 VLP) contained more unstable VLP than the VLP purified by cation-exchange chromatography (cHPV16 VLP). In addition, hHPV16 VLPs more strongly react with monoclonal antibody (Camvir-1) that reacts with linear epitope of HPV16 L1 than cHPV16 VLPs do. Therefore, two kinds of VLPs showed different assembly characteristics. In the study of mouse immunization, the hHPV16 VLP induced weak or no spleenocyte proliferation while the cHPV16 VLP effectively induced spleenocyte proliferation. In the humoral immune responses, the neutralizing antibody titer induced by cHPV16 VLP was three times higher than that induced by hHPV16 VLP while the total antibody titers against HPV16 VLP induced by two kinds of VLPs were similar. These immune response results indicate that the conformational change on the VLP by interaction between VLP and heparin on column resin results in alteration of immune responses. Therefore, we suggest that the selection of ligand in purification of HPV16 VLP is an important factor for determining the quality of VLP as a prophylactic vaccine.

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P-404: The Detection of HPV Genotypes in Anal Cancer Biopsy Specimens from Sydney, Australia

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Background: HPV type 16 is the commonest worldwide genotype associated with anal cancer. We describe the distribution of HPV genotypes found in anal cancer specimens from Sydney, Australia. Methods: Cases of anal cancer were identified from databases of participating hospitals. Paraffin blocks were retrieved from storage and slices obtained. Histological confirmation of the diagnosis was made from adjacent slices and the central slice analysed for the presence of HPV. Amplification of HPV was performed using L1 consensus primers PGMY09-PGMY11. Genotyping of 27 human papillomavirus types was performed by a single-hybridization, reverse line blot detection method.
Results: 114 biopsy specimens were tested. 18 different genotypes were detected, 3 specimens had no HPV detected and one had an unknown type. 69 (60.5%) had HPV16 alone, with a further 15 (13%) contained HPV16 and at least one other genotype. Type 18 was found in only 4 cases, two of which also contained type 16. HPV6 was the next most common, occurring alone in 7 (6%) and with other HPV types in 3 cases.
Conclusions: A wide variety of HPV genotypes was detected. 96 (84%) contained at least types 16 or 18. However, a significant minority (16%) contained only HPV types not covered by currently available prophylactic vaccines. This may have implications for future vaccine developments.

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P-407: Outcome of Conservative Surgical Management of Superficially Invasive Squamous Cell Carcinoma of the Anus

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Objective: Combined modality therapy (CMT) is the standard of care for anal cancer, effectively treating early-stage cancer, but with significant morbidity. As high-risk patients are increasingly being screened and followed, an increasing number of patients with asymptomatic, superficially invasive squamous cell carcinomas of the anus (SISCCA) are being identified. The goal of this study was to determine the outcome of morbidity-sparing local excision of SISCCA.
Methods: SISCCA was defined as superficially or "microinvasive" or not specified, but without invasion of the muscularis. Clinically suspicious areas were excised and high-grade anal intraepithelial neoplasia (HGAIN) was ablated. Lesions were re-excised, if indicated. Those with margins positive for carcinoma were referred for CMT. High-resolution anoscopy (HRA) was performed every 4 months and recurrent HGAIN or cancer aggressively treated locally. Patients were referred for CMT if recurrent cancer was unresectable.
Results: 43 patients were diagnosed with SISCCA (9 intra-anal, 34 peri-anal): 34 HIV-positive (33 men, 1 woman) and 9 HIV-negative (5 men, 4 women), median age 49 years (range: 33-75). No patients died of anal cancer or required a colostomy after a median follow-

up of 43 months. 30 of 43 (70%) developed HGAIN post-excision. 24 of 43 (56%) required multiple excisions. 21 were treated in-office for recurrent HGAIN with infrared coagulation including 5 with >3 procedures. 10 (23%) developed recurrent cancer. 6 (12%) were re-excised and only 4 of 43 (9%) required CMT. All achieved a complete response. At last follow-up, 3 died of unrelated causes, 27 (63%) had no evidence of HGAIN or anal cancer, 8 (19%) had HGAIN, and 5 (12%) were recently treated for HGAIN. **Conclusions:** Surgical excision of SISCCA combined with close follow-up using HRA and ablation of recurrent HGAIN or SISCCA produces an excellent outcome, sparing most patients the morbidity of CMT.

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P-410: Episomal and Integrated HPV-16 Loads in Anal Intraepithelial Neoplasia in HIV-Seropositive Men

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Background: The natural history of episomal and integrated viral loads in the course of anal HPV-16 infection has not been reported in HIV-positive men having sex with men (MSM). The aim of this study was to investigate the association between these biomarkers and progression of HPV-16 infection to anal intraepithelial neoplasia (AIN).
Methods: Of the 247 HIV-positive MSM followed prospectively for 3 years in the Human Immunodeficiency and Papilloma VIrus Research Group (HIPVIRG) cohort, 135 (54.7%) provided 665 HPV-16-positive anal samples. AIN grade was assessed on biopsies during periodical high-resolution anoscopies. Episomal and integrated HPV-16 loads were measured with quantitative real-time PCR assays. HPV-16 integration was confirmed with DIPS-PCR to demonstrate the presence of viral-cellular junctions.
Results: HPV-16 DNA in anal samples was exclusively episomal in 627 samples (94.3%), exclusively integrated in 9 samples (1.4%), and of mixed physical state in 22 samples (3.3%). The average HPV-16 load was 892 copies/cell (median: 24, range: 0-31,289). Episomal load [odds ratio (OR)=1.5, 95% confidence interval (CI)=1.1-2.1], number of HPV types [OR=1.4, 95% CI=1.1-1.8] and current smoking [OR=4.8, 95% CI=1.3-18.6] were associated with high-grade AIN (AIN-2,3) after adjusting for age and CD4 counts. Integrated load was not associated with AIN-2,3 [OR=1.1, 95% CI=0.4-1.1]. Samples with higher episomal load were less likely to contain integrated HPV-16 forms [OR=0.5, 95% CI=0.3-0.8]. Integration was detected equally in the absence of AIN, in AIN-1 and in AIN-2,3, suggesting that it occurs in the course of infection independently of AIN. Integration was confirmed in 19 (61.3%) of 31 specimens by DIPS-PCR. The analysis of viral-cellular junctions showed that integration sites within host genome were fortuitous. **Conclusions:** High episomal HPV-16 load is predictive of AIN-2,3 while integration does not seem to be a factor involved in the progression to pre-cancerous lesions.

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P-411: Recurrence of High Grade Anal Intraepithelial Neoplasia and Cancer in Patients Treated with Combined Modality Therapy for Anal Cancer

Naomi Jay[1], J. Michael Berry[2], Teresa Darragh[3], Joel Palefsky[1]

Objectives: Combined modality therapy (CMT) consisting of chemotherapy and radiation is the standard of care for anal cancer treatment, with recurrence rates reported to be > 16%. Identification of high-grade anal intraepithelial neoplasia (HGAIN) precursor lesions through high resolution anoscopy (HRA)-guided biopsy followed by ablation may prevent recurrent anal cancer in CMT-treated patients and early-stage recurrent cancer may possibly be treated with local excision. Follow-up of patients' post-CMT may therefore be important. This study sought to determine prevalence of HGAIN and cancer following completion of CMT for anal cancer.
Methods: Retrospective chart review of anal cancer patients diagnosed since 1999 and treated with CMT, referred to the University of California San Francisco for post-CMT follow-up.
Results: 52 men and 14 women completed CMT for anal cancer and were evaluated with HRA post-treatment. The average age at cancer diagnosis was 53.2 years (range 37-66). Nineteen (29%) patients returned for one visit and 47 (71%) returned for on-

	N	Post-CMT HGAIN N (%)	Post-CMT Cancer N (%)
HIV-positive men	39	8 (21%)	7 (18%)
HIV-negative men	12	5 (42%)	2 (17%)
HIV status unknown, male	1	0	0
HIV-positive women	0	0	0
HIV-negative women	14	1 (7%)	0
Total	66	14 (21%)	9 (14%)

going HRA every 4-6 months (Figure). HGAIN was diagnosed in 14 (21%) patients (average 29 months post-CMT) and cancer in 9 (14%) patients (average 28 months post-CMT). 54% of HGAIN and 78% of cancers were diagnosed at the first post-CMT evaluation (range 7-47 months). Twelve of 14 HGAIN were treated; 5 of the 12 treated lesions progressed to cancer despite treatment. Seven of 9 patients with recurrent cancers were treated surgically, half with colostomy-sparing local resection; one was lost to follow-up and one died with the cause of death unknown. **Conclusions:** One-third of patients had HGAIN or cancer post-CMT treatment. These were more common in men suggesting they be targeted for post-CMT HRA evaluation. It is unclear if detection and treatment of HGAIN will prevent cancer recurrence. Early follow-up and intervention may lead to better outcomes, including the possibility of curative local colostomy-sparing resection.

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P-412: Prevalence and Associations of Anal Human Papillomavirus (HPV) Infection in African-Caribbean (AC) women in Toronto, Canada

Anuradha Rebbapragada[1,2], Robert Remis[3], Lisungu Chieza[4], Stephen Perusini[1], Juan Liu[3], Mona Loutfy[3,6], Scott Boerner[7], Megan Saunders[4], Wangari Tharao[4], Rupert Kaul[5,8]

Objectives: The association of anal cancer with HPV infection is well established; however, little is known about the epidemiology of anal HPV infection in women and its associations with cervico-vaginal HPV, host immune status and demographic factors. We examined anal HPV type distribution in HIV-infected and HIV-uninfected women.
Methods: An ongoing cross-sectional study at the Women's Health in Women's Hands Community Health Centre is recruiting HIV-infected and HIV-uninfected AC women residing in Toronto. At a single study visit, women completed a detailed ACASI0 based socio-behavioural questionnaire, provided blood for serology (HSV1/2, CMV, Hepatitis, syphilis and HIV) and urine for gonorrhea and chlamydia testing. Self-administered vaginal and anal swabs were collected for Gram stain, HPV testing (Hybrid Capture2 and Linear Array on all) and anal PAP.
Results: The analysis to date includes 230 HIV-uninfected and 94 HIV-infected women. Anal HPV was detected in 58.7% of HIV-infected and 24.2% of HIV-uninfected women. Anal high risk HPV (HR HPV) infection was detected in 43.5% of HIV-infected and 10.3% of HIV-uninfected women. HPV68 was the most common anal HR HPV type in both groups (10.9% in HIV-infected and 3.1% in HIV-uninfected). In vaginal samples, HPV 58 was most common in HIV-infected women (12.9%) and types 16/18/51 were most common in HIV-uninfected women (4.9% for each). Anal infection with any HPV type, any HR HPV type, multiple (>3) HR HPV types and co-infection with HSV2 were all associated with HIV infection ($p<0.0001$ for all). High and low grade squamous intra-epithelial lesions (HSIL and LSIL) were found in 5.5% and 7.7% of HIV-infected and in 0.0% and 0.9% of HIV-uninfected women. **Conclusions:** These findings highlight the urgent need to better characterize anal HPV infection in women and, in particular, the determinants HPV dissemination across ano-genital sites and the development of anal malignancies.

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P-413: Prevalence and Associations of Vaginal Human Papillomavirus (HPV) Infection in African-Caribbean (AC) Women in Toronto

Anuradha Rebbapragada[1,2], Robert Remis[3], Lisungu Chieza[4], Stephen Perusini[1], Juan Liu[3], Sanja Huibner[5], Megan Saunders[4], Mona Loutfy[3,6], Wangari Tharao[4], Rupert Kaul[5,7]

Introduction: Variations in HPV types across different regions and sub-populations may not reflect the pooled national estimate for HPV prevalence. Epidemiologic studies are needed to elucidate the associations between HPV type distribution, cervical cancer, ethnicity, sexual behaviour and host biological determinants in specific populations. We sought to examine these associations in immigrant women from AC countries residing in Toronto, Canada.
Methods: A cross-sectional study at the Women's Health in Women's Hands Community Health Centre is recruiting HIV-infected and HIV-uninfected AC women. At a single study visit, women completed a detailed ACASI0 based socio-behavioural questionnaire, provided blood for serology (HSV1/2, CMV, Hepatitis, syphilis and HIV) and urine for gonorrhea and chlamydia testing. Self-administered vaginal and anal swabs were collected for Gram stain, HPV testing (Hybrid Capture2 and Linear Array on all) and anal PAP.
Results: The analysis to date includes 230 HIV-uninfected and 94 HIV-infected women. HPV and high risk (HR) HPV infection was detected in 81.7% and 59.1% of HIV-infected and 40.8% and 24.2% of HIV-uninfected women, respectively. HPV 58 was most common in HIV-infected women (12.9%). HPV 16, 18 and 51 were most common in HIV-uninfected women (4.9% for each). Infection with any HPV type, any HR HPV type, multiple HR HPV types (>3) and co-infection with HSV2 were associated with HIV infection ($p<0.0001$ for all). In HIV-uninfected women, HPV prevalence was associated with younger age (33.9% in 15-24 years versus 21.1% in women >55 years, $p=0.012$), number of sex partners (16.5% with none versus 46.7% with >2, $p=0.026$) and smoking (18.5% never versus 54.8% with any smoking history, $p<0.0001$).
Conclusions: The high rates of HPV infection and distinct pattern of type distribution emphasize the need to assess the type-specific risk for cervical cancer and the proportional impact of HPV vaccines in this population.

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P-414: Performance of HPV Testing and Anal Cytology to Detect High-Grade Anal Intraepithelial Neoplasia in Men Who Have Sex with Men

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Objectives: Anal cytology is used in clinical settings to screen for human papillomavirus (HPV)-related anal intraepithelial neoplasia (AIN) and cancer in high-risk populations. Because of inherent test performance limitations with cytology, anal HPV testing may potentially be used to screen for high-grade AIN (HGAIN-AIN 2/3). We compared performance of cytology and HPV testing to detect HGAIN in HIV-positive and HIV-negative men who have sex with men (MSM). **Methods:** Baseline cross-sectional analysis of 302 HIV-positive and 176 HIV-negative MSM in a prospective cohort study of AIN. Participants underwent testing for HPV DNA with L1 consensus PCR, conventional cytology, and high-resolution anoscopy (HRA)-guided biopsy if indicated. Abnormal cytology and histology definitions were: atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and HGAIN, respectively. The gold standard was based on histology and HRA findings. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for anal cytology and HPV testing categories: oncogenic HPV infection (positive versus negative), HPV-16 (positive versus

Table 1: Performance of HPV testing and cytology to detect AIN 2/3, HIV-positive MSM (n=302)

	% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
HPV testing alone				
Oncogenic HPV+	79 (71-84)	37 (29-46)	61 (54-68)	58 (47-69)
≥3 HPV types	73 (65-79)	43 (35-52)	62 (54-68)	56 (46-65)
HPV-16+	50 (42-58)	77 (68-84)	73 (64-81)	55 (48-63)
Cytology ≥ ASC-US	87 (81-91)	42 (33-51)	65 (59-71)	72 (60-81)
Cytology ≥ ASC-US and Oncogenic HPV+	84 (77-88)	47 (38-56)	67 (60-73)	70 (59-79)
Cytology ≥ ASC-US and ≥3 HPV types	83 (77-88)	46 (38-55)	66 (59-72)	69 (58-78)
Cytology ≥ ASC-US and HPV-16	83 (76-88)	51 (43-60)	68 (61-74)	70 (60-79)
Cytology ≥ LSIL	77 (70-83)	53 (44-62)	67 (60-73)	65 (55-74)
Cytology ≥ LSIL and Oncogenic HPV+	72 (64-79)	63 (54-71)	71 (63-77)	64 (55-72)
Cytology ≥ LSIL and ≥3 HPV types	71 (64-78)	62 (53-70)	70 (63-77)	63 (54-71)
Cytology ≥ LSIL and HPV-16	61 (53-69)	80 (72-86)	79 (71-86)	62 (54-69)
Cytology = HSIL	46 (39-54)	93 (87-97)	89 (80-95)	58 (51-65)

Table 2: Performance of HPV testing and cytology to detect AIN 2/3, HIV-negative MSM (n=176)

	% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
HPV testing alone				
Oncogenic HPV+	54 (34-73)	70 (62-77)	24 (14-37)	90 (82-94)
≥3 HPV types	38 (21-59)	79 (71-85)	24 (13-40)	88 (81-93)
HPV-16+	31 (15-52)	88 (81-92)	31 (15-52)	88 (81-92)
Cytology ≥ ASC-US	65 (44-82)	81 (74-87)	38 (24-53)	93 (87-97)
Cytology ≥ ASC-US and Oncogenic HPV+	58 (37-76)	84 (77-89)	38 (24-55)	92 (86-96)
Cytology ≥ ASC-US and ≥3 HPV types	58 (38-76)	85 (78-90)	39 (24-57)	92 (86-96)
Cytology ≥ ASC-US and HPV-16	58 (38-76)	85 (78-90)	39 (24-57)	92 (86-96)
Cytology ≥LSIL	50 (30-70)	85 (78-90)	37 (22-55)	91 (84-95)
Cytology ≥ LSIL and Oncogenic HPV+	42 (24-63)	90 (84-94)	42 (24-63)	90 (84-94)
Cytology ≥ LSIL and ≥3 HPV types	42 (24-63)	90 (84-94)	42 (24-63)	90 (84-94)
Cytology ≥ LSIL and HPV-16	35 (18-56)	95 (90-98)	56 (31-79)	89 (83-94)
Cytology = HSIL	27 (12-48)	98 (93-99)	70 (35-91)	89 (82-93)

negative), infection with >= 3 HPV types versus 1-2 types.
Results: Among HIV-positive MSM, 71% had oncogenic HPV infection, 56% had HGAIN. Among HIV-negative MSM, 34% had oncogenic HPV infection, 15% had HGAIN. Among HIV-positive MSM (**Table 1**), cytology (>=ASC-US) was most sensitive for HGAIN; however oncogenic HPV testing alone demonstrated comparable sensitivity and specificity. HPV-16 testing alone or as an adjunct to cytology (LSIL or worse) was highly specific for HGAIN, but sensitivity was reduced. In HIV-negative MSM (**Table 2**), cytology (>=ASC-US) was more sensitive for HGAIN compared to HPV testing. HPV testing as an adjunct to cytology did not significantly improve test performance in this population.
Conclusions: Cytology remains the preferred screening method for HGAIN. In settings where HPV testing is employed, HPV-16 status may be useful to triage high-risk individuals for HRA.

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P-415: DNA Methylation Profiling Across the Spectrum of HPV-Associated Anal Squamous Neoplasia

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Introduction: There is growing evidence to suggest that changes in host genome DNA methylation patterns are among the critical molecular alterations associated with HPV-related carcinogenesis in squamous cell cancers (SCC) of the cervix and oropharynx. However, there is very little known about the epigenetic changes associated with the development of anal SCC. We sought to characterize HPV genotype and broad methylation profiles across the spectrum of anal squamous neoplasia.
Methods: Thirty-one formalin-fixed paraffin embedded samples from 25 patients were evaluated and included normal anal mucosa (NM; n=4), SCC-in situ (SCC-IS n=11) and invasive SCC (n=16). SFP10 LiPA HPV-typing system was used to determine the HPV status. Bisulfite-modified DNA was interrogated for methylation at 1,505 CpG loci representing 807 genes using the Illumina GoldenGate Methylation Assay.
Results: Our population was comprised of 13 women and 12 men with a median age of 48 years (range 26-81). Five patients were immunocompromised either by HIV or chemotherapy. All patients demonstrated infection with at least one high-risk HPV subtype, with HPV 16 noted in 15/16 patients with SCC. There was a trend towards increasing percentages of total CpG loci methylated with histologic progression; 56 ± 4% for NM, 61 ± 4% for SCC-IS and 63 ± 1% for SCC (p=NS). Fourteen gene loci were found to be significantly and differentially methylated (Kruskal-Wallis $p<0.01$) across the 3 groups, with nine genes associated with disease progression (**Figure 1**).
Conclusion: In HPV-associated anal neoplasms, we have identified a panel of methylated genes associated with the progression from anal NM to SCC. To our knowledge, this is the first reported application of broad high-throughput methylation analysis to anal neoplasia. Our findings have future implications for advances in the understanding of HPV-associated carcinogenesis as well as for anal SCC screening, diagnosis and treatment.

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GENE	FUNCTION	p-Value
FRK	Growth regulation at the G1/S transition; Stabilizes PTEN	0.0026
HOXA5	Growth regulation; Upregulates p53 expression	0.0062
KRT1	Epidermal differentiation (Known HPV target)	0.0038
PADI4	Cell cycle control through upregulation of p53; Apoptosis inducer	0.0071
S100A2	Tumor suppressor required for proper keratinocyte differentiation	0.0050
TGFB3	Tumor suppressor in epithelial cancers; silenced in Bowen's disease	0.0021
TNFRSF10B	Transduces an apoptosis signal in response to TNF and FADD	0.0061
DIO3	Degradation of thyroid hormones	0.0064
FLT1	Mediator of VEGF signaling	0.0079

P-435: An HPV Genotyping Assay that Utilizes QIAGEN Hybrid Capture Technology

Ha Thai[1], Wen Chen[1], Tanya Gay[1], Sarah McLeod[1], Dana Pfister[1], Irina Nazarenko[1]

Background: The digene HPV Genotyping PS Test (PS)(TM) was developed for the specific detection of HPV 16, 18, and 45.
Objectives: To assess the initial performance of the digene HPV Genotyping PS Test using both plasmids and clinical specimens using different specimen collection media types.
Methods: The PS Test is a non target amplification assay based on Hybrid Capture® technology. The test utilizes QIAGEN's proprietary hybrid-specific antibodies for the detection of HPV DNA targets. Analytical specificity of the PS Test HPV 16, 18, and 45 probes was evaluated using a panel of both high-risk and low-risk HPV plasmids. Performance of the PS Test was compared to type specific quantitative-PCR (qPCR) to evaluate concordance. Concordance to qPCR was demonstrated using both cervical brush samples in Specimen Transport Media, (STM) and liquid based cytology samples.
Results and Conclusions: The digene HPV Genotyping PS Test specifically detects HPV 16, 18, and 45 with at least 5000 copies sensitivity. The assay is highly specific and has been shown to have no cross-reactivity with a panel of other high-risk or low-risk HPV types present at up to 108 copies per reaction. Initial performance on clinical specimens was demonstrated on STM, PreservCyt, and SurePath® cervical specimens that were initially screened by the HC2 assay. Performance capability and accuracy of the test was compared to sequence specific qPCR for HPV 16, 18, and 45 with overall concordance between the two detection methods shown to be greater than 95%. In addition, the compatibility of the PS Test with STM, PreservCyt and SurePath specimens was demonstrated. The applications presented here are for research use only. Not for use in diagnostic procedures.

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P-436: Multicenter Evaluation of the Cobas 4800 HPV Test: Detection of CIN2+ Lesions Compared to hc2

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Background: Many studies have demonstrated that high risk (HR)-HPV testing is more sensitive than cytology in the detection of CIN2+ lesions. Many methods have been proposed to detect the presence of HR-HPV in cervical samples, with the Hybrid Capture 2 (HC2) system (Qiagen) being the most widely used. In this multicenter study we have evaluated the prototype cobas 4800 HPV test , for the detection of CIN2+ lesions. The prototype cobas® 4800 is a highly automated system that performs sample preparation, real-time HR-HPV amplification and simultaneous detection of 12 HR-HPV genotypes in a single pool, with separate detection of HPV16, HPV18, as well as the human beta globin gene, in a single tube.
Methods: From May till October 2008, aliquots of specimens from 1340 consecutive patients from three clinical centers for whom a HC2 test was requested were stored for further analysis with the prototype cobas 4800 HPV test. HC2, cobas 4800 HPV, and histology/biopsy results were available for 408 patients. Samples with discrepant HPV test results were analyzed with the Linear Array HPV Test (LA). Clinical disease (CIN2+ and CIN3+) was determined as the highest categorization of pathology observed from histology results.
Results: Among the 408 patients undergoing colposcopy with a biopsy, 160 were found to have a CIN2 lesion or greater; 94 with a CIN3 lesion or greater. Among the CIN2+ samples, 140 samples were concordant positive and 11 concordant negative. We observed 9 hc2 positive/cobas4800 negative CIN2+ samples: 6 samples were HR-HPV positive by LA analysis, whereas 3 samples were LA low-risk HPV positive.
Conclusion: This multicenter evaluation of the prototype cobas 4800 HPV test in comparison with hc2 showed 94% concordant results in samples from patients with CIN2+ lesions. Few samples gave discordant results and further analysis is ongoing.

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P-437: Can HPV DNA/mRNA and/or p16 Status Reduce the Need for Biopsies in Pregnant Women with Atypical Cytology?

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Background: Atypical cervical cytology is common in pregnant women. Cancer is uncommon but must be excluded. Colposcopy is difficult and punch biopsies may fail to detect microinvasive cancer. Conisation can be hazardous.
Objective: To evaluate if HPV genotyping, HPV mRNA analysis and/or p16 analysis can facilitate detection of microinvasive cancer in pregnant women with atypical cytology.
Methods: We investigated 132 women: 25 non pregnant, 26 pregnant with normal cytology, 30 non pregnant, 26 pregnant women with atypical cytology and 25 women with invasive cancer. Samples were taken for analysis of HPV DNA genotype (in house real-time PCR and Linear array, Roche), HPV E6/E7 mRNA (in house real-time PCR and PreTect HPV proofer, NorChip), p16 immunocytochemistry (mtm laboratories). In house genotyping assays for HPV DNA and HPV mRNA were performed with TaqMan real-time PCR targeting E6/E7of HPV 6, 11, and 12 highrisk-types including 16,18.
Results: Among women with normal cytology, 10 (40%) of non-pregnant women were p16 positive and 11(44%) were HPV DNA positive but only 2 (8%) mRNA positive; 4 (15%) of pregnant women were p16 positive, 11 (42%) HPV DNA positive and 3 (12%) mRNA positive. Among women with atypical cytology 25 (83%) of the non pregnant women were p16 positive, 28 (93%) HPV DNA positive, 23 (77%) mRNA positive; and 21 (81%) of the pregnant women were p16 positive, 25 (96%) HPV DNA positive and 19 (73%) mRNA positive. Pregnant women with verified CIN3 +, including one case of

microinvasive cancer were all identified by both p16 and E6/E7 mRNA in house real-time PCR and non pregnant CIN3 with either p16 or E6/E7 mRNA. Only 2 women with cancer were negative for DNA, additionally 1 negative for mRNA but all were p16 positive.

Conclusion: Pregnancy does not increase p16 positivity.A combination of p16 analysis and HPV E6/E7 mRNA in house real-time PCR may be a useful, non invasive tool to identify pregnant women with atypical cytology at risk of having an invasive cancer, thus reducing the number of biopsies needed. Confirmative studies are needed.

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P-438: Expression of Rap1 and P16INK4A in Cervical Preneoplastic and Neoplastic Lesions Associated with High Risk Human Papillomavirus Infection

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Background: High risk human papillomavirus (HPV) plays a central role in the development and progression of cervical cancer. The neoplastic progression occurs through the action of E6 and E7 viral oncoproteins which cause dysregulation of cell cycle proteins such as pRB, p53 and p16INK4A and other cell cycle regulatory proteins. The search for cervical cancer biomarkers is still crucial to enhance diagnostic consistency and reproducibility of cervical cancer. Rap1 is a member of the Ras family of small GTPases that is activated by diverse extracellular stimuli in many cell types. Either a defective or an excess in Rap1 activation has been reported to contribute to malignancy via distinct biological effects in different cell types.

Objectives: To analyze and compare expression patterns of Rap 1, a potential biomarker for cervical cancer, to the p16 INK4A by means of immunohistochemical analysis.

Methods: Immunocytochemical analysis of p16INK4A and Rap 1 was performed on 57 cervical biopsies classified as follows: 9 normal, 22 LGSIL, 13 HGSIL and 13 cervical carcinoma (CC). Staining intensity was assessed using a 0-3 scoring system. HPV was detected by PCR and specific HPV typing was done. The Wilcoxon and Fisher exact tests were employed for the statistical analysis.

Results: Unlike p16INK4A, Rap1 expression was upregulated in the LGSIL group associated with high risk HPV infection. Both markers, Rap1 and p16INK4a, were found overexpressed in the HGSIL and CC lesions.

Conclusions: Rap1 GTPase may be a biomarker for predicting the progression of HPV-related squamous intraepithelial lesions and a combination of Rap1 GTPase/p16INK4a expression seemed to be useful to those identified as high grade and invasive lesions. Our data suggests that Rap1 is a good marker for predicting cervical cancer progression.

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P-439: Biological Activity of Rare HR-HPV in Cervical Cancer

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Background: Evidence for active involvement of HPV-16 and other frequent HR-HPV in cervical squamous cell carcinoma (C-SCC) has been provided in many studies. However, evidence for biological activity of rare (prevalence <1.5%) HR-HPV in C-SCC and putative (p)HR-HPV is limited.

Methods: We analyzed 68 formalin-fixed paraffin-embedded (FFPE) C-SCC tissues for HPV DNA and RNA expression. HPV DNA was detected by a semi-quantitative multiplex genotyping (MPG) assay enabling detection of 54 mucosal HPV types. HR-HPV DNA positive samples were analyzed for the expression of E6*I transcripts by a sensitive, short (65bp) amplimer RT-PCR assay developed for 13 HR- and 1 pHR-HPV. Expression of cellular proteins p53, pRb and p16INK4a as surrogate markers for active HPV transformation was investigated by immunohistochemistry (IHC) in 29 C-SCC.

Results: All 68 biopsies yielded analyzable DNA and RNA. Sixty-seven (99%) contained HPV sequences of which 54 (81%) were single and 13 (19%) multiple infections. Type-specific prevalences were: HPV-16 (67%), -31 (9%), -18 (7%), -33, -35, -45 (6%), -82 (4%), -52 (3%), -39, -51, -56, -58, -59 and pHR-HPV-26, -66 (1%). E6*I-RNA was present in 32/34 (94%) HPV-16 single, all other HR-HPV single infections and one single pHR HPV-66 case. In all multiple infections RNA of at least 1 type was present. IHC pattern typical for HPV-induced transformation with up-regulated p16 and down-regulated pRb was found in 27/29 (93%) cases while p53 was down-regulated in 23 of these 27 (85%).

Conclusions: This pilot study shows that rare HR-HPV types 35, 56, 51, 39 and pHR HPV66 are actively contributing to the transformed phenotype of individual C-SCC. The E6*I-RNA assay is suitable for FFPE material and is a valuable tool to understand the role of rare HR-HPV in C-SCC.

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P-450: Randomized Controlled Trial of Primary HPV tTesting in a Canadian Organized Screening Program

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Objectives: The HPV FOCAL trial is the first North American randomized controlled trial examining the efficacy of hr-HPV

DNA testing as a stand-alone screening test followed by liquid based cytology (LBC) triage of hr-HPV-positive women compared with LBC followed by hr-HPV triage with >=CIN3 as the outcome.

Methods: This randomized controlled trial will recruit 33,000 women aged 25 to 65 who are assigned to 1 of 3 study arms- Control: LBC testing. Negatives screen again at 2 and 4 years. Colposcopy referral at >LSIL or ASCUS+HPV-positive. Safety-Check: HR-HPV testing. Exit screen at 2 years with LBC. HPV-positives undergo reflex cytology testing and managed same as intervention arm. Intervention: HR-HPV testing. Negatives exit screen at 4 years. HPV-positives undergo reflex cytology testing. Exit colposcopy referral at ASC-US threshold or HPV-positive. Outcome measures: Confirmed >=CIN3 detected at exit screen in control and intervention arms; confirmed >CIN2 in control arm at 2 years, safety arm at exit; clearance of HPV infection in HPV-positives at recruitment.

Results: By March 1, 2010, results were available for 11,729 women. Demographics equally distributed in the 3 arms indicating successful randomization. Control: 94.4% LBC negative; 0.9% had high grade squamous intraepithelial lesions (HSIL) on LBC, and highest HSIL rates in those 25-29 yrs (2.9%). Safety and Intervention: 91.9% and 92.1% hr-HPV negative respectively. Highest hr-HPV positivity rate in those 25-29 yrs (23.8% and 25.2%) and lowest in those 60-65 yrs (3.0% and 3.0%). Preliminary colpscopy and pathology rates are presented (Figure 1).

Discussion: The HPV Focal trial is the first randomized controlled trial to compare liquid based cytology with HPV triage vs HPV with LBC triage as the primary screen for cervical cancer. Over 11,000 women have been recruited and preliminary results show successful randomization.

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	Screen Negative (%)	Colposcopy Referral Rate	Pathology Results		
			CIN2	CIN3	CIN2/3
Control (LBC)	3687/3907 (94.4)	3.4%	19/112 (17.0)	11/112 (9.8)	30/112 (26.8)
Safety Arm	3626/3947 (91.9)	3.0%	19/100 (19.0)	16/100 (16.0)	35/100 (35.0)
Intervention Arm	3579/3875 (92.4)	3.0%	22/104 (21.2)	18/104 (17.3)	40/104 (38.5)

P-461: Digital Analysis of IHC images for Detecting HPV E6/E7 Oncoproteins in CIN2/3 and Cervical Cancer Tissues

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Background: Overexpression of E6/E7 in CIN2/3 results in malignant transformation of cervical epithelium. Scarce reports show direct detection of E6/E7 oncoproteins in cervical tissues due to limited anti-E6 or E7 antibody.

Objectives: 1) To demonstrate the overexpression and localization of E6/E7 proteins in CIN2/3, SCC, and cervical adenocarcinoma by IHC using our novel anti-E6 or E7 antibodies. 2) To digitize the IHC slides and to use automated image analysis software to quantitate stains based on color, intensity, and staining pattern. 3) To compare and correlate the automated image analysis results to those obtained from standard microscopic scoring.

Methods: Two monoclonal anti-E6 and two monoclonal anti-E7 antibodies were used to perform IHC on a total of 12 tissue microarrays that contain previously-identified paraffin-embedded tissue cores including 120 CIN2, 120 CIN3, 48 SCC, 48 adenocarcinoma and their normal epithelial counterparts. IHC staining for each core on the tissue microarrays was scored by a gynecologic pathologist as percentage of cells with nuclear and cytoplasmic staining. Staining of adjacent dysplastic and normal epithelia was determined for each core for comparative purposes. Image analysis was performed with a combined nuclear+cytoplasmic algorithm on selected regions of interest from each core on the same IHC stained tissue microarrays following digitization on BioImagene's iScan digital slide scanner (Sunnyvale, CA). Correlation of image analysis results to manual microscopic scores was established.

Preliminary Results: Dysplasia and cancer show distinguishable staining compared to normal adjacent epithelium. Both cytoplasmic and nuclear staining were found in the dysplastic cells, with increasing staining percent positivity seen for CIN2, CIN3, and cervical cancers respectively. Image analysis can provide more quantitative information on staining intensity and percent positivity relative to manual microscopic scoring. Determination of specific thresholds for intensity and positivity for E6/E7 may provide an aid to the pathologist in the evaluation and grading of dysplasia.

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P-463: Comparison of the Roche cobas 4800 vs. Qiagen hc2 Tests for Detection of High-Risk HPV DNA

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Objective: To compare the performance of the Roche cobas 4800 vs. Qiagen hc2 tests for detection of high-risk (HR) HPV DNA.

Methods: ThinPrep cervical specimens from females enrolled in

the HPV FOCAL Trial (ISRCTN79347302) were tested for HR HPV by Qiagen hc2 High Risk HPV DNA Test (hc2) and Roche cobas 4800 HPV (cobas 4800). All specimens were also tested by Roche Linear Array HPV Genotyping Test (LA).

Results: For 806 specimens, overall agreement between hc2 and cobas 4800 was 96.4% (κ=0.77; 95% CI 0.68-0.85). hc2 and cobas 4800 were both positive for 53 specimens (**Table 1**); by LA, 52 were HR HPV positive and 1 was low risk (LR) HPV (CP6108). Of the 724 hc2/cobas 4800 negative specimens, by LA, 714 were negative or had only LR HPV; however, 3 were HPV 16, 1 was HPV 18 and 6 were other HR HPV (non-HPV 16 or 18). Overall, 15 specimens were hc2 positive/cobas 4800 negative and 14 were hc2 negative/cobas 4800 positive; LA results for these discordant specimens are shown in **Table 2**. The majority of the discordant specimens displayed signals close to their respective assay detection threshold. Of the HR HPV genotypes detected by LA, cobas 4800 was positive for 17/20 (85.0%) HPV 16; 6/7 (85.7%) HPV 18; and 41/49 (83.7%) other HR HPV; overall genotype agreement between LA and cobas 4800 was 84.2%. An additional 2,000 specimens will be tested over the next month.

Conclusions: Agreement between the hc2 and cobas 4800 results was high. Based on LA as a reference standard, the hc2 test had more false positive HR HPV detections than cobas 4800, and cobas 4800 detected more HR HPV than hc2.

Table 1	Qiagen hc2		
Roche cobas 4800		+	-
	+	53	14
	-	15	724

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Table 2 Linear Array Results for Discordant hc2/cobas 4800 Specimens		
	N	Linear Array
hc2 + /cobas 4800 -	15	7 HPV negative; 6 LR HPV; 2 HR HPV (non-HPV 16 or 18)
hc2 - /cobas 4800 +	14	5 HPV 16; 1 HPV 18; 1 HPV 16 and 51; 7 HR HPV (non-HPV 16 or 18)