WHO HPV LabNet - Newsletter 06

WHO HPV LabNet
World Health Organization's
Global Human Papillomavirus (HPV)
Laboratory Network

Preface: This newsletter aims to provide a brief and updated overview of the WHO HPV LabNet activities, this being the 6th edition of the 6-monthly newsletter.

Content:

1. HPV LabNet – Announcements

VLP Validation Studies Identify HPV 16 and HPV 18 VLP Preparations Suitable for Use by WHO HPV LabNet in Serology Assays

- High-quality VLPs for use in ELISA assays are prerequisite for running HPV serology studies. As there are no commercial sources of VLPs and most laboratories are not able to produce their own, the unavailability of this essential reagent has hindered the implementation of standardized HPV serology assays for use in epidemiological studies. Such studies are crucial for the **WHO HPV LabNet** for monitoring vaccine impact and guiding vaccination programs. One of the highest priorities of the WHO HPV LabNet is the provision of quality-assured VLPs to all of its member laboratories in order to improve assay performance and inter-laboratory comparability of antibody measurements.
- Following an open call for donations of moderate amounts of purified VLPs on the WHO website and in the LabNet newsletter during Dec 2008- Feb 2009, 5 laboratories donated VLPs to the WHO HPV LabNet for use in ELISA. The donated VLPs were characterized by the 2 GRLs and NIBSC. GRL/Sweden conducted an initial characterization of the donated VLPs and concluded that most of the VLP preparations were of good quality. The HPV 16 and 18 VLPs with best results and sufficient quantity were evaluated in ELISA tests conducted by all WHO HPV LabNet members.
  The quality control (**QC**) and coating conditions of HPV VLPs used in these immunoassays will be published in the upcoming WHO HPV Laboratory Manual.

- **The HPV 16 and 18 VLPs are now available to the WHO HPV LabNet members for intended use.** The process for requesting these preparations is being finalized and will be announced in the near future.

Currently Available WHO Standards *

1st International Standard (IS) for anti-HPV 16 serum (product no. 05/134)

- This **IS** will serve as the primary biological standard for antibodies to HPV 16
- It may be used in immunoassays utilizing VLPs and neutralization tests utilizing PsVs of adequate sensitivity

1st IS for HPV type 16 DNA (product no. 06/202) & 18 DNA (product no. 06/206)

- These **IS**s should be used to calibrate in-house or working standards for the amplification and detection of HPV types 16 & 18 DNA

*Available through order from the National Institute for Biological Standards and Control (**NIBSC**), UK, http://www.nibsc.ac.uk/products/product_catalogues.aspx.

The **4th WHO HPV LabNet HPV DNA Proficiency Panel** will be distributed mid-2010, following advertisement and call for applications in April 2010.

- The WHO sought global participation in this international HPV DNA typing proficiency study.
- Laboratories that are or will be involved in HPV surveillance and/or vaccine development were most welcomed, with participation in the study being voluntary and free of charge.
- The panel is composed of 43 samples (purified whole genomic plasmids) of low/high-risk HPV types in a background of human DNA, and 3 extraction controls. Samples will include single and/or multiple types at varying concentrations traceable to established or candidate **ISs** to evaluate sensitivity and type-specificity of detection.
- Laboratories using more than one 'typing assay' are encouraged to test the samples using each assay.

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2. An Update: HPV LabNet International Collaborative Studies

International Collaborative Study to Assess a Candidate WHO 1st International Standard for Anti-HPV 18 Human Serum

HPV serology is essential for both HPV vaccinology and epidemiology. Comparability and reliability of serological data requires international standardisation, particularly in terms of antibody units. WHO has, in collaboration with National Institute Biological Standards and Control (**NIBSC**), established the first IS for anti-HPV 16 serum. The WHO HPV LabNet Global Reference Laboratory (**GRL**), Sweden, in collaboration with the Regional Reference Laboratory (**RRL**), Thailand, has now obtained **candidate material for preparing the 1st WHO IS for antibodies to HPV 18**. The candidate material is sera collected from two healthy women in Thailand who participated in an epidemiological study on HPV conducted by the National Cancer Institute (**NCI**) of Thailand. The donated sera have been assayed by the GRL, Sweden and have been shown to be mono-specific for HPV 18 antibodies with a high OD titre.

In June 2010, NIBSC will fill and freeze-dry the pooled sera and an international collaborative study will be organized. HPV laboratories that have established neutralization assays and/or immunoassays will be invited to participate. The aims of this collaborative study are to:

- Assess the suitability of the freeze-dried serum to serve as the 1st IS for antibodies to HPV 18 with an assigned unitage in International Units (**IU**) per ampoule.
- Characterize the candidate IS in terms of reactivity and specificity using a range of immunoassays and neutralization assays performed in different laboratories.
- Establish the extent to which the candidate IS is suitable to serve as a standard for a variety of different samples being assayed.

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The 3rd WHO HPV LabNet HPV DNA Proficiency Panel, 2009

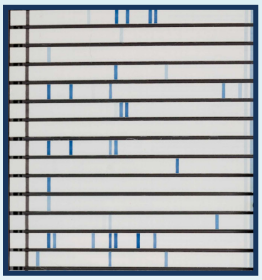
The 3rd WHO HPV DNA proficiency panel, produced by GRL/Sweden, was distributed within the WHO HPV LabNet. This panel provided the possibility to analyze specificity and sensitivity for different HPV typing assays, in terms of correctly identifying 14 high-risk HPV types and 2 low-risk HPV types, deemed the most important for HPV surveillance and monitoring.

Objectives:

The primary aims of this study were to:

- Assess the proficiency of HPV typing assays used within the LabNet;
- Assess whether there had been improvement in laboratories, not proficient last year;
- Identify any problems with assays routinely performed.

Methods:



- The WHO HPV DNA proficiency panel was composed of 43 samples, which were all purified plasmids diluted in a background of human placenta DNA.
- Alterations from the previous (2nd) panel were:
  - A new clone of HPV 39 was used that could be detected by assays based on the PGMY primer system;
  - For HPV 68, two subtypes were included, since the prototype HPV 68A could not be detected by PGMY primers.
- HPV types included in the panel were: 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68A and 68B.
- Three samples: A, B and C, were cell lines used as DNA extraction controls.

Results:

A summary of the HPV genotyping assays employed included:

- Two laboratories tested the panel using two different assays;
- The PGMY based blot assay (PGMY-CHUV), described in the WHO HPV manual, was used by five laboratories;
- The commercial HPV Linear Array Test (Roche) was used by four laboratories;
- Two laboratories used Luminex-based assays with modified GP primers;
- A type-specific PCR and GP-based blot assay was used by one laboratory, respectively.

- The new plasmids of HPV 39 and 68B were detected by most laboratories, whereas HPV 68A could not be detected by PGMY-based assays (as expected).
- Six laboratories were 100 % proficient for all HPV types tested according to LabNet criteria.
- One laboratory did not detect any false positive samples but could not detect HPV 39 and 56 using PGMY-CHUV (proficient for 15/17 types: 88% proficiency) or for HPV 39, 59 and 68 using GP5+/6+ RHB.
- Three laboratories had two or three false positive results and these datasets were considered as not proficient.
  - Of these, one laboratory could detect all HPV types whereas the others could not detect HPV 56 and 39, respectively.
- One laboratory had 6 false positive results and was also not able to detect HPV 16, 45 or 51.
- The extraction control included in the panel could be correctly identified in the highest copy number (2500 HPV 16 genomes per 5ul) by all laboratories, except one.

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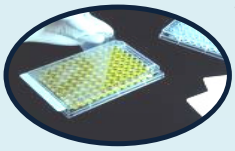
Phase 2 HPV LabNet Study on HPV Serology (VLP-ELISA): The 1st WHO HPV LabNet International Proficiency Study of HPV Serology

Background:

HPV serology is essential for both HPV vaccinology and epidemiology. VLP-ELISA correlates well with the neutralization assay but is easier to implement. Comparability and reliability of serological data requires international standardization, particularly in terms of antibody units and cut-off levels, as well as international proficiency testing of serological laboratories.



Objective:

The WHO HPV LabNet global proficiency study of HPV serology had the following goals:

- Proficient serological testing methodology established at all HPV LabNet labs;
- Inter-lab variation determined; consistency of performance investigated;
- International "cut-off" criterion agreed;
- Perform an evaluation using an international collaborative study on an assay intended to be described in WHO HPV Lab Manual as suitable for large scale serology in HPV seroepidemiology and monitoring of HPV vaccination.

Material and Method:

The WHO HPV LabNet agreed on a common standard operating procedure (**SOP**) for ELISA based on consensus from collaborative studies. The protocol was first tested by all LabNet members in terms of robustness, unambiguity and stability and a second version of the SOP established. VLPs were donated from 5 different sources and the most stable, sensitive and specific preparation was selected for common use and distributed to WHO LabNet members as coated plates. A blinded challenge proficiency panel of 52 serum samples from PCR verified HPV-infected women and 11 virginal women was tested in parallel with the WHO HPV 16 IS serum to report data in IUs.

Results:

Ten laboratories in 6 WHO Regions participated. Proficiency criteria for detection of HPV 16 antibodies were a sensitivity of at least 50% and a specificity of 100%. It was not possible to use a common cut-off in terms of IU (see example with data with a 4 IU cut-off in Table 1), but a mean value of the results from the control sera plus three standard deviations gave optimal performance (Table 1). Six of ten participating laboratories were proficient for HPV 16 antibody testing.

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Conclusion:

International HPV serology proficiency studies are feasible.
Common SOPs, IU's and cut-off criteria have been established.

Table 1. HPV 16 antibody positivity using different cut-off levels.

	HPV 16 DNA positive women n=52 (%)		Virginal women n=11 (%)	
	Previously Assigned [4,0 IU]	Individual based on virgin sera [mean + 3SD]	Previously Assigned [4,0 IU]	Individual based on virgin sera [mean + 3SD]
Lab 1	24 (46)	34 (65)	0	0
Lab 2	25 (48)	22 (42)	1 (9)	0
Lab 3	25 (48)	27 (52)	0	0
Lab 4	21 (40)	30 (58)	0	0
Lab 5	20 (38)	36 (69)	0	0
Lab 6	26 (50)	30 (58)	0	0
Lab 7	26 (50)	36 (69)	0	0
Lab 8	32 (61)	10 (19)	5 (45)	0
Lab 9	24 (46)	23 (44)	0	0
Lab 10	23 (44)	22 (42)	1 (9)	0

3. WHO HPV LabNet Training Workshops

WHO HPV LabNet Training Workshop on HPV Genotyping and HPV Serology Laboratory Performance
15-18 March, 2010

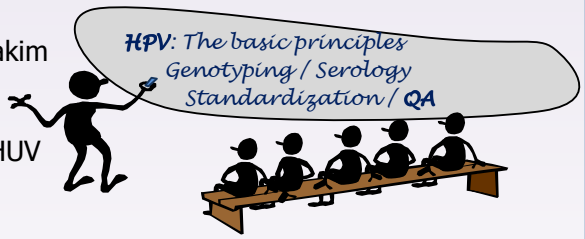
WHO provides technical support to countries in building up HPV laboratory testing capacity to support HPV surveillance

HPV laboratory surveillance and vaccination impact monitoring is a critical element in the process of HPV vaccine introduction. WHO established the HPV LabNet to harmonize and standardize laboratory testing procedures to support consistent laboratory evaluation of regional disease burden and monitor the performance of HPV vaccines (http://www.who.int/biologicals/vaccines/hpv/en/index.html). Significant progress has been made in the area of standardization of HPV laboratory testing to promote international harmonization including development of ISs, evaluation and standardization of HPV assays, development of Quality Assurance (QA)/ QC scheme, capacity building and training. Requests for technical support on HPV laboratory testing have been received from countries.

In response to these requests, WHO/HQ organized the **2010 WHO HPV LabNet Training Workshop on HPV Genotyping and HPV Serology Laboratory Performance.** This was hosted by the HPV LabNet RRL, Europe, Institute of Microbiology, Centre Hospitalier Universitaire Vaudois (**CHUV**)**, Lausanne, Switzerland.**

Objectives:

- Provide training on basic/general knowledge of HPV biology and vaccines; the concept of international standardization; theoretical knowledge and practical performance of HPV genotyping and serology assays; principles and knowledge of HPV laboratory QA/QC; and to share experience of WHO HPV LabNet.
- Technical facilitation was offered by Prof. Joakim Dillner, Dr. Elizabeth Unger, Dr. Morag Ferguson, Dr. Iwao Kukimoto, Prof. Denise Nardelli-Haefliger, Dr. Roland Sahli and CHUV staff.



- The workshop was attended by 15 participants from 12 countries.
- Trainees were nominated by WHO Regional Offices from countries active in HPV laboratory testing to support HPV surveillance; namely **Argentina, India, Iran, Italy, Morocco, Republic of Korea and Uganda**.
- Training included all aspects involved in HPV laboratory testing, and laboratory performance of proficient HPV genotyping and serology assays verified by the WHO HPV LabNet.
- A comprehensive HPV Laboratory Manual (to be published) and critical materials for setting up a new assay were provided to participants to take back to help set up basic assays.
- The training was regarded as very informative, helpful and in good timing when they are setting up the HPV laboratory testing capacity in the countries to support HPV epidemiological studies.
- Implementing good performance of HPV laboratory testing and international standardization will ensure that proficient and standardized assays are used in HPV epidemiological studies and reliable data are generated to promote international harmonization of HPV laboratory testing and comparability of data cross different laboratories. It is anticipated that participants will be able to assist the HPV LabNet RRLs to provide technical support to other laboratories in the respective region, when such need is identified.

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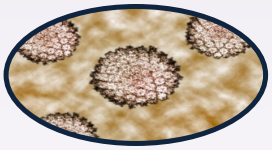
PAHO/WHO HPV Training Workshop
26-28 April, 2010

The HPV LabNet RRL, Pan American Health Organization (PAHO) region, recently conducted a successful **1st PAHO/WHO Training Workshop on HPV Genotyping Laboratory Performance** at the National Institute of Infectious Diseases-ANLIS "Dr. Malbran", **Buenos Aires (Argentina)**.

- Support and facilitation from: WHO/HQ (Geneva), WHO/PAHO (WDC), PAHO Argentina, and National Institute of Infectious Diseases-ANLIS "Dr. Malbran" (Buenos Aires, Argentina);
- Target audience: scientists, technicians and public health personnel working or interested in developing HPV assays (particularly supporting HPV surveillance and vaccine impact monitoring);
- Attendants: **20 participants from both local HPV laboratories (Argentine Natl. Lab. Network) and Latin American countries (Brazil, Colombia, Costa Rica, Cuba, Chile, Ecuador, El Salvador, Guatemala, Mexico, Nicaragua, Panama, Trinidad & Tobago, Uruguay and Venezuela)**.

Objectives:

- Provide practical and theoretical training on HPV genotyping testing to personnel from Hospitals/Institutes/Centres concerned with cervical cancer in Latin American countries (**AMR**) in order to standardize and harmonize HPV testing in the region to support HPV surveillance and vaccination impact monitoring;
- Disseminate knowledge on HPV and HPV vaccines, ISs/reference reagents, QC/assurance of assays, and HPV surveillance;
- Identify candidate national reference laboratories and start organizing the regional network on the basis of the experience acquired in the organization of the Argentine HPV LabNet;
- Share experiences from national laboratories in providing laboratory support to HPV surveillance and vaccination monitoring; identify gaps for further support.



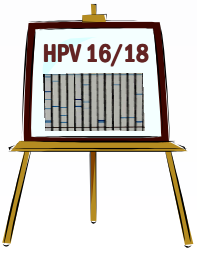
Workshop program:

Theoretical component:

- Basic information about HPV infection, pathogenesis, carcinogenesis, HPV vaccines, HPV surveillance and vaccination impact monitoring;
- Overview of HPV genotyping assays and their application;
- WHO strategies in facilitating HPV vaccine introduction, international standardization, role/activities of HPV LabNet, QA/QC of HPV laboratory testing;
- Introduction of the HPV Laboratory Manual, HPV proficiency and confirmatory testing.

Practical component:

Participants were divided in two groups (2 laboratories) to promote active and personal work. Practical classes included:



- DNA extraction from clinical samples (exfoliated cells, fresh tissues and paraffin embedded biopsies);
- HPV detection and genotyping was performed with HPV typing by MY09/11 PCR-Restriction Fragment Length Polymorphisms and GP5+/6+ PCR-Reverse Line Blotting Hybridization on 37 patient samples (4 negative, 33 positive, including multiple infections) and CaSki DNA HPV16 positive;
- Results and/or problems were discussed.

Results:

- All participants showed great interest and good management of molecular techniques, whilst the level of knowledge on HPV and the experience in HPV assays was considerably varied. Theoretical classes fulfilled the purpose of harmonizing these aspects.
- At the end of the activity, participants stated that they felt very motivated and greatly appreciated the opportunity to take advantage of practical classes through a hands-on approach.
- The course was found to be well organized, very gripping, helpful and user-friendly.
- Trainers were considered well-prepared and responsive to the participants' learning needs.

Conclusion:

- The Workshop provided the first opportunity to gather professionals from Latin American academic and public-health institutions, interested in approaching HPV infection and cervical cancer, from the laboratory surveillance perspective.
- Experience exchange was very rich, both from the scientific-technical and logistic perspective.
- When returning to their countries, and based on the information received, participants will analyze the possibilities to start and/or proceed with their activities in the field of HPV, now with the possibility of interacting with the HPV LabNet, under the WHO guidelines.
- This activity confirms once again the relevance of integrating scientific knowledge with each country's health related realities.

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4. HPV LabNet Regional Reference Laboratory Activities: A Snapshot

RRL, WHO South-East Asia Region

HPV LabNet RRLs actively participate in a number of tasks associated with HPV detection and surveillance in and around their geographical location. Examples of such activities (over a 6-month period) within the NCI, Thailand are:

- Establishment of a HPV DNA/serology project to determine baseline data regarding the prevalence of HPV types and antibodies present in Thai women;
- Organization of a Training Workshop on HPV genotyping and HPV 16/18 serology, conducted on 03-07 August, 2009 at the NCI, Bangkok, Thailand;
- Participation in the 3rd HPV DNA Proficiency Test (2009);
- Participation in the 1st HPV LabNet Proficiency Study of HPV 16 serology (Phase 2 HPV LabNet study on HPV serology: VLP-ELISA);
- Blood collection for developing International Standardization of Reference Sera and the 1st HPV Antibodies Standardization to Global WHO HPV LabNet (Sweden) and NIBSC;
- Training on HPV genotyping for Medical Staff from Bhutan, 19 March – 14 April, 2010;
- Collaborative work (preliminary-study) with Hospital in Bhutan on HPV typing for indicating baseline of HPV subtype in Bhuthamese women;
- Contribution to HPV LabNet news especially "Call for participation of the 2010 WHO HPV LabNet Proficiency Study of HPV DNA Typing" to the audience who attended the Training Workshop on HPV genotyping and HPV 16/18 serology.

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With active support from the Ministry of Health and Family Welfare, Government of India and Indian Council of Medical Research; the Institute of Cytology and Preventive Oncology (ICPO) promotes basic biological and clinico-epidemiological research in the field of cancers most prevalent in India with an emphasis on early detection, and primary and secondary prevention.

ICPO has made significant contributions in the field of cervical cancer and HPV and is a recognized:

- National Reference Centre for HPV and cervical cancer;
- WHO Collaborating Centre for Research and Training in Cytology;

HPV research / diagnostics

ICPO laboratories provide or are involved in:

- Specialized diagnostic referral services at a national level;
- Active training in HPV diagnostics, while supporting research for medical/scientific degrees in HPV molecular epidemiology;
- HPV surveillance studies in early cancer detection camps as part of HPV prevalence studies;
- Molecular and genetic studies to determine the prevalence of, and factors, affecting HPV infection in the cervix and other organ sites (e.g. oral cavity and oesophageal tissues);
- Clinical trials of potential anti-HPV therapeutics possessing the capability to eliminate HPV DNA from the cervix;
- Clinical trials of the Gardasil HPV vaccine (Merck), in India;
- A demonstration project for the FastHPV/CareHPV test (in collaboration with other centres).

Other research activities

- We actively participate in HPV-LabNet initiatives to improve the quality of laboratory services for effective surveillance and HPV vaccination monitoring in South-East Asia.
- Participate in LabNet International Collaborative HPV Studies coordinated by NIBSC (UK).
- Perform in-house qualitative and quantitative PCR for major high-risk and low-risk types.
- We have the capacity to perform techniques like HC2, multiplex PCR (L1-based, HPV 16 / 18), sequencing and HPV variant analyses.
- We have established the PGMY-reverse line blot assay (with CHUV Switzerland), as a more efficient method for comprehensive genotyping.
- We have made major advances in establishing HPV serology and set-up of VLP-ELISA for HPV16 and HPV18.
- Participation in confirmatory testing and testing proficiency panels (Global HPV LabNet) to gain proficiency in detection and typing of various high-risk and low-risk HPVs;
- We have strengthened the QA/QC program existing in the laboratory (with WHO support).

HPV meetings / communication

- We contributed to the organization of a workshop and practical course on HPV genotyping and HPV16/18 serology held in NCI – Thailand, for the South-East Asian Region;
- We play a key role in disseminating information (i.e. personal communication and presentations of LabNet activities in conferences and symposia) from WHO to various laboratories in the region engaged in HPV research and diagnostics.
- We contributed to the development of the Global HPV Laboratory Manual;
- We are putting in efforts for the development of an Indian HPV Vaccination Registry, which will be beneficial in monitoring HPV vaccine response in India.

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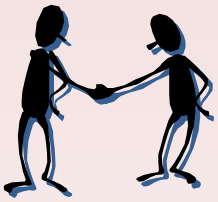
Vaccination has not been introduced into the public sector in South Africa. As such, our HPV LabNet efforts are centred on molecular epidemiology to provide supporting data for HPV vaccine introduction. We work with a number of groups to provide HPV typing and prevalence data in HIV-positive women to inform policy on cervical screening. There have been requests from African laboratories for training.

Recently received approval for establishment of a Molecular Epidemiology Laboratory within the National Health Laboratory Service (NHLS) diagnostic facility, including HPV LabNet functions, to open in mid-2010, led by Prof. Anna-Lise Williamson.

HPV vaccination-based research

- We are involved in HPV testing (HC2) of participants in a GSK-sponsored trial entitled: "A phase 1/2 partially-blind randomised, controlled study to assess the safety and immunogenicity of GlaxoSmithKline Biologicals' HPV16/18 VLP AS04 vaccine administered intramuscularly according to a three dose schedule (0, 1, 6 month) in human immunodeficiency virus (HIV) infected female subjects aged 18-25 years".
    - Specimens received from European & Developing Countries Clinical Trials Partnership project for HPV typing.
- We have had requests to type HPVs in adolescent specimens obtained from a high prevalence HIV cohort (Durban).
- A new project on genital warts has been initiated, with HPV typing to be performed in the new Molecular Epidemiology Laboratory.



Collaborative research studies

- In 2009, we continued working with research groups around the country including Pretoria, Durban; and most closely, Johannesburg:
    - We have trained staff at the STI Reference Centre, National Institute for Communicable Diseases (NICD), Johannesburg, to perform HPV typing, which is now done on a continual basis;
    - Established collaboration between our laboratory and the STI Reference Centre (NICD) to detect anti-HPV antibodies and type genital HPVs in heterosexual men with anogenital warts, male urethral discharge and asymptomatic men. The aim was to determine HPV genotypic distribution among men presenting with anogenital lesions. Valuable information will be attained with regards to HPV type prevalence, and present/previous exposure to HPV, as determined by oral and serum HPV-specific antibodies, among men in South Africa.

Other research studies

- Our laboratory has continued working on HPV in couples. A total of 409 couples were enrolled in the study, 122 were both HIV-negative, 101 were both HIV-infected, 186 were HIV-discordant (144, with only women HIV-positive; 42, with only men HIV-positive).

HPV meetings

- We have made presentations at several meetings where members of the South African Government's National Department of Health have co-attended.
- Gave a talk entitled "The impact of HIV infection on the transmission of human papillomavirus in heterosexual couples" at the Stop Cervical Cancer Meeting (July 2009) in Somerset West, attended by some of the First Ladies of Africa including President Zuma's wife, Tobeka Madiba-Zuma. She has since made cervical cancer one of her projects, which has been acknowledged in the National Department of Health.
- We were part of the organising committee of a meeting "HPV Research Agenda in HIV Prevalent Regions" (Dec 2009) co-sponsored by IAVI, RHRU and DTHF, attended by interested parties including people from the National Department of Health.

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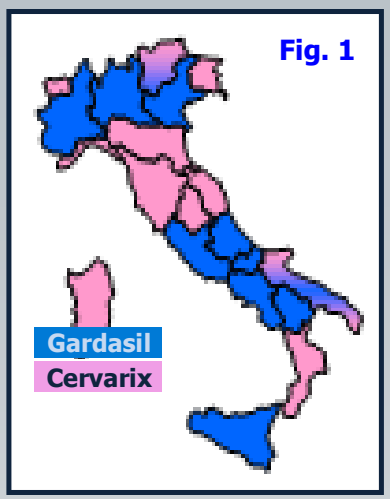
5. Country Experiences: HPV Research/Diagnostic Laboratories

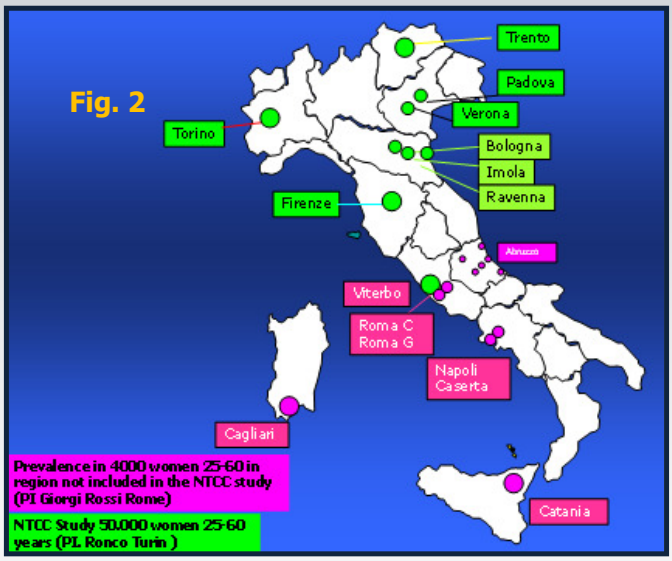
ISPO Cancer Prevention and Research Institute – Florence, Italy

The ISPO Cancer Prevention and Research Institute has participated in the HPV LabNet since June 2007. In 2008, the Italian Ministry of Health launched a mass HPV 'free-of-charge' vaccination 'of 11-year old girls. Quadrivalent and bivalent vaccines were adopted unevenly throughout the Italian regions on the basis of regional public tender (Fig. 1).



Our laboratory has coordinated HPV testing and typing across 4 HPV prevalence surveys financed by the Minister of Health to evaluate HPV distribution in Italy, prior to vaccination (Fig. 2):

- The NTCC study: HPV prevalence and type distribution in over 50,000 women (25-60 years old) participating in the Italian screening program for cervical cancer;
- Pregio-1 and Pregio-2 studies: HPV prevalence and type distribution in 4,000 young women (18-24 years old) across 10 different Italian regions;
- Typing of 1,000 ≥CIN2 lesions from tissue samples obtained in Italy over the past 10 years.



The combined data was considered representative of the entire country, given the same methodology was used across 10 geographical regions. GP5+/GP6+ PCR-based assay targeting a 150bp region of the L1 gene. The preliminary data on HPV distribution among the general population (18-60 years old) showed a typical HPV infection prevalence curve, with a peak at age 21-24 years and no statistical difference between Northern, Central and Southern Italy. HPV 16, followed by 31, 51, 56, 58, 52, were the most prevalent HPV types in the screening population.

Among the ≥CIN2 samples, cases were ascertained through the electronic databases of pathology units, with paraffin-embedded tissue samples collected from historical archives and tested accordingly. The proportion of cancers due to HPV 16/18 decreased with age at diagnosis [92% in women <35y to 73% in women >55y].

The laboratory is also involved in HPV serological studies. The randomized study 'Effective surveillance and impact of HPV vaccination on screening for cervical cancer', sponsored by public health, will evaluate:

- HPV 16/18 antibodies levels and HPV type distribution at enrolment and at next round of screening;
- Comparison of HPV prevalence in cervical samples and urine to evaluate the possibility to monitor HPV status in younger girls.

Serological and HPV typing assay procedures ascertained at the recent WHO HPV LabNet workshop in Lausanne (15-18 March, 2010) will be applied within the laboratory.

Other involvements include:

- Contribution to the dissemination of knowledge and use of HPV IS reagents throughout Italy to improve typing accuracy and serological measurements;
- Co-ordination within the Italian Group of Cervical Cancer Screening to include all Italian HPV testing laboratories within QA programs;
- A pilot project of assessing the feasibility of HPV testing in screening programs:
  - We will co-ordinate HPV testing QA, cytology and organization of a Bio-Bank for cervical samples collected in the study;
- Participation in:
  - HPV LabNet Collaborative Studies on development of WHO IS for HPV DNA testing;
  - International QA programmes
  - Contribution to the HPV LabNet Manual;

The HPV LabNet has been very important in supporting the development of HPV laboratories within resource-poor countries, while at the same time facilitating sharing with more experienced laboratories within industrialized countries, thereby aiding their entry as national reference laboratories.

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CHU (Centre Hospitalier et Universitaire) – Reims, France

Unit of Molecular and Cellular Biology located within a Pathology laboratory. Our centre performs:

- Routine hospital diagnostic screening:
  - Molecular pathology and molecular genetics;
  - Primary cervical screening since 1997 (Pap smear and HPV test by HC2), with a "Reims' cohort" of >30,000 women.

- Basic research, with an involvement in:
  - Head and neck tumours (plasticity of epithelial cells / role of HPV in this process).
  - Clinical research of HPV in cervix and oropharyngeal cancers.
  - Genotyping of women with persistent HPV infection to improve their follow-up (specifically HPV 16, 18, 45, but also other HR-HPV).
  - Detection of HPV 16 viral load, physical status of HPV (mainly HPV 16), viral expression (E6/E7 mRNA), and cell ploidy.
  - We are involved in the Canceropôle Grand-Est (Canceropole of Great-East of France: web of the 5 regions and the 5 CHU of the French Great-East) linked to Germany, in "Applied Tumour Virology", and in industrial applications about HPV diagnosis and vaccination.
  - Hospital grants for the study of molecular markers (HPV in oropharyngeal cancers).

- Involvement in teaching of cellular biology at the Faculty of Medicine, Reims.
- Participation in national training on screening for cervical tumours for gynaecologists, pathologists, and biologists.
- We recently created (2008) a French association for the study of HPV and Polyomavirus infections (AFIPP: Association Francophone pour l'étude des Infections par Papillomavirus and Polyomavirus), with each team offering an annual AFIPP meeting in France to improve communication especially between students and potentially to prepare grants.

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6. Meeting Updates

4th Biennial Conference of AOGIN

New Delhi, India
26-28 March, 2010

More information can be obtained on the link: http://www.aoginindia.org

Theme: "Agenda 2010: Towards eradication of cervical cancer".
Meeting for scientists, pathologists, virologists, clinicians, gynaecologists, gynaecologic oncologists, epidemiologists, medical students, postgraduates and health administrators to come together and discuss the latest advances in the field of cervical cancer prevention.

- Record number of 1050 participants from 50 countries attended the Conference
- 152 abstracts were presented on a range of topics:
  - Cervical cancer etiopathogenesis, prevention by HPV vaccination, treatment of preneoplastic and neoplastic lesions, advocacy and education.

A highlight meeting was the presentation of the AOGIN Guidance for Cervical Cancer Prevention, which is currently under preparation (final version to be soon published).

There were 3 pre-congress workshops (to enhance practical skills), on 26 March, 2010:

- Colposcopy and Hands-On LEEP workshop, facilitated by Dr. Jeffrey Tan;
- Cytology and HPV testing Workshop, facilitated by Prof. Suzanne Garland;
- Oncosurgical Video Workshop, facilitated by Dr. Michael Quinn.

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WHO HPV Vaccine Advisory Committee (HVAC) meeting

WHO Headquarters, Geneva, Switzerland
27-29 April, 2010

Meeting Objectives:

The WHO HPV Vaccine Advisory Committee (HVAC) convened with several goals:

- Review new information relevant to WHO policy on HPV Vaccine;
- Receive update on ongoing HPV vaccination efforts and research;
- "wrap up" HVAC in its current form and assist colleagues as the HPV Vaccine transitions from IVR to Immunization, Reproductive Health, and Cancer Control.

New Clinical Data Reviewed:

- Both licensed vaccines have excellent efficacy in preventing CIN 2/3 from HPV 16/18 with efficacy data of 6.4 years for the bivalent, 5 years for the quadrivalent, and out to 9.5 years with a monovalent HPV 16 prototype vaccine.
- Both vaccines demonstrate partial protection against oncogenic HPV type 31. The bivalent vaccine also demonstrates partial protection against HPV types 33 and 45. Long-term monitoring is needed to understand the public health significance of these findings.
- Continued follow up of trials and post-marketing surveillance indicate that both vaccines appear safe and do not cause adverse outcomes.

- Co-administration of HPV vaccines with some common adolescent vaccines appears safe and does not impair immunogenicity to any antigen examined.
- The manufacturer of the bivalent vaccine sponsored a multicenter study to compare the immunogenicity of the bivalent vaccine to the quadrivalent vaccine in females 18-45 years old. The serum neutralizing antibody response measured using a pseudovirion-based neutralization assay (PBNA) was higher for the bivalent than the quadrivalent vaccine for both HPV 16 and 18 in every age group through 24 months of follow up. The clinical relevance of this is unclear. There is as yet no immune correlate of protection defined.
- Two doses of the quadrivalent vaccine given at 0 and 6 months in girls (9-13 years old) yields non-inferior antibody responses to HPV-16,-18,-6,-11 at month 7, as compared to both a 3-dose regimen in women (16-26 years old) and a 3-dose regimen in girls (9-13 years old). Whether the antibody response in the two dose regimen will remain non-inferior as time from vaccination increases is under study.
- Studies of quadrivalent vaccine in males naïve to HPV types 6, 11, 16 and 18 demonstrated 90% efficacy against extra genital lesions in all males and 77% against anal intra-anal neoplasia in men who have sex with men.
- In HIV seropositive females, both vaccines appear safe. The quadrivalent vaccine is immunogenic in seropositive males and females.
- Demonstration projects in low income countries show that HPV vaccine is generally acceptable and tolerated, and that high coverage can be achieved in school and non-school based programs.
- In Australia, where high coverage of the quadrivalent vaccine in females (12-26 years old) has been achieved, a significant decrease in warts in young women (~50% reduction), as well as a lesser but significant reduction in young men, is now being demonstrated; the latter suggesting the presence of herd immunity.

HPV Vaccine Policy Developments Reviewed:

- The WHO Position Paper was published in April, 2009 and both vaccines have been prequalified.
- WHO regards the HPV vaccine as an important component of a comprehensive cervical cancer prevention program.
- The Global Alliance for Vaccines and Immunization (GAVI) has fully endorsed the HPV vaccine to be included in its portfolio. Currently, GAVI has a funding shortfall. However, GAVI would like to prepare for introduction so that when funding is available, applications will be allowed.
- WHO recently published The Report of the Meeting on HPV Vaccine Impact Coverage and Monitoring, http://whqlibdoc.who.int/hq/2010/WHO_IVB_10.05_eng.pdf
    - Monitoring HPV vaccine impact is complex and is not a prerequisite to initiating vaccination
    - Endpoints of HPV prevalence in women soon after sexual debut could be conducted in one or two select settings as an early marker of vaccine impact
    - Cervical cancer is the primary disease endpoint and all countries should consider establishing or strengthening cervical cancer registries

Future HPV Vaccine Considerations

- At this time, data is lacking to recommend any changes in HPV vaccine policy to SAGE. However, data will need to be assimilated and reviewed in an ongoing manner.
- An internationally recognized entity will be needed to ensure laboratory proficiency for HPV testing in vaccine clinical trials and monitoring, to promote implementation of ISs, in order to ensure reliability and comparability of data across worldwide labs.

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7. Upcoming Meetings

26th International Papillomavirus Conference & Clinical Workshop

Montreal, Canada
03-08 July, 2010

More information can be obtained on the link: http://hpv2010.org/main

Informal WHO HPV LabNet Symposium: Laboratory testing in HPV vaccinology and HPV surveillance

Montreal, Canada - at IPV Conference
05 July, 2010 (*provisionally scheduled*)

Objective: To review and discuss the ongoing activities in the HPV LabNet as well as in national HPV surveillance reference laboratories, including a series of short presentations."

- Co-organized by Prof. Joakim Dillner and Dr. Elizabeth Unger, GRLs of WHO HPV LabNet

8. Useful Web Links

- **http://www.who.int/biologicals/vaccines/hpv/en/index.html**
- **http://www.who.int/hpvcentre/en**
- **http://www.who.int/immunization/en**
- **http://www.who.int/biologicals/en**
- **http://www.nibsc.ac.uk/products/biological_reference_materials.aspx**
- **http://www.ipvsoc.org/index.html**
- **http://www.iarc.fr**
- **http://www.uicc.org**
- **http://www.eurogin.com/index.html**
- **http://www.aogin.com/pages/index.php**

9. Contributions for Next Edition of HPV LabNet Newsletter

Please forward suggested contributions within the next four months to the Co-Editors of the HPV LabNet Newsletter. We welcome local initiatives; pertinent projects; prevalence data for HPV DNA, especially genotype specific sero-surveillance; etc. Contributions are sought from the wider global HPV community. Suggestions on avenues for wider dissemination of the newsletter are welcome.

The HPV LabNet Newsletter is published 6-monthly in English by:
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