



WHO HPV LabNet - Newsletter 03



World Health
Organization

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 3rd edition of the 6-monthly newsletter.

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1. Follow-up: WHO HPV LabNet 2008 Priorities

The two main areas of priority for WHO HPV LabNet in 2008 were Capacity Building and Standardization of assays for HPV DNA and serology. In addressing these:

- The HPV Global Reference Laboratory in Sweden has led a 2nd proficiency study for HPV DNA genotyping and a collaborative study on HPV16 serology (direct VLP-ELISA) (**see Section 3**).
- The HPV Regional Reference Laboratory in Switzerland is currently evaluating results/feedback from a recent technology transfer of a non-commercial HPV genotyping assay (general PGM1 primer PCR with genotyping by reverse hybridization) in the HPV LabNet, to be reported in the 4th edition of this newsletter.

These studies aimed at ensuring all HPV LabNet Regional laboratories are capable of performing HPV DNA genotyping and serology testing. This included evaluating and improving suggested standard operating procedures (**SOPs**) following assay experience, to help in development of an HPV Laboratory Manual.

- The National Institute for Biological Standards and Control (**NIBSC**), as a WHO International Laboratory for Biological Standards, have been extensively engaged in developing International Standards (**IS**) and reference reagents for use in assays for detecting either HPV DNA or HPV antibodies (**see Section 4**).

At the WHO 59th Expert Committee on Biological Standardization (**ECBS**) meeting, October 13th-17th 2008, Geneva, Switzerland:

- First ISs for HPV16 and HPV18 DNA were adopted;
- New proposals for HPV-related projects were approved:
 - Panel of HPV16 and HPV18-specific monoclonal antibodies for quality control of HPV VLPs used in immunoassays;
 - First IS for HPV18 antibody;
 - DNA panel for HPV types 31, 33, 45, 52 and 58.

These new projects will be pursued in the coming year(s).

2. Call for Contributions

To facilitate the progress towards assay harmonization and standardization, HPV LabNet is continuing studies on HPV serology.

**WHO has opened calls for donation of qualified VLPs
and well-characterized serum samples
for use in HPV LabNet collaborative studies**

Call for contribution of: *HPV type 16 and 18 L1-based Virus-Like Particles*

Meaningful and comparable HPV antibody measurements are dependent upon serology assays that use high quality, intact L1 VLPs that display type-specific conformational epitopes. These VLPs also form the basis for current vaccine formulations. As there are no commercial sources of VLPs and most laboratories are not able to produce their own VLPs, the unavailability of this essential reagent is hindering the implementation of standardized HPV serology assays for use in epidemiological studies. Such studies are crucial 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.



To achieve this goal, WHO is seeking contributions of relatively modest quantities of HPV type 16 and/or type 18 VLPs from academic, government and commercial laboratories worldwide.

- Approximately 1 mg of each preparation will be sufficient to conduct initial inter-laboratory comparisons of antibody measurements in order to begin to address the impact of lot-to-lot variation of assay components, VLP stability, and optimal method of VLP transfer (i.e. coated plates versus protein suspensions).
- The VLPs must be free of complex proprietary issues and be allowed to be transferred to participating laboratories worldwide with a minimum of paperwork.
- NIBSC, a WHO International Laboratory on Biological Standards, will be responsible for the receipt, storage and distribution of the donated VLPs as well as the organization of the studies.
- Data generated from the WHO HPV LabNet studies will become the property of WHO and may be analyzed for publication by the HPV LabNet either as an internal document or peer-reviewed manuscript.
- All results will be handled in an anonymous fashion. WHO will ensure that linking data to originating laboratories will be kept confidential.
- Donors will be duly acknowledged by the WHO and the HPV LabNet for the kind donation of this critical reagent. The WHO HPV LabNet will provide data on all comparisons to donors if requested.

For further information about the donation and additional technical information, please contact **Dr. Dianna Wilkinson** at dwilkinson@nibsc.ac.uk. In order to proceed with this project in a timely manner, the WHO HPV LabNet would greatly appreciate responses from prospective donors by the end of **January, 2009**.

Call for contribution of: *Human serum reference samples for HPV serology*



The global establishment of meaningful and comparable HPV antibody assays is an important priority task of the WHO HPV LabNet. Availability of high quality, standardized HPV serology is a crucial component for the progress of HPV vaccine research. With the aim of improving assay performance and inter-laboratory comparability of antibody measurements, one of the highest priorities of the WHO HPV LabNet is the provision of proficiency panels composed of well-characterized serum samples to all of its member laboratories.

To achieve this, WHO is seeking contributions of relatively modest quantities of human serum reference samples from academic, government and commercial laboratories worldwide, specifically we seek:

- Serum samples from patients who have histopathologically verified cervical cancer or cervical intraepithelial neoplasia grade 2 or 3, where the tumor has been conclusively demonstrated to contain either HPV16 DNA or HPV18 DNA;
- Serum samples from women reporting no sexual experience or from children 2-10 years of age;
- The serum samples should be available in amounts of 4 milliliters or more;
- The serum samples must be collected with appropriate Institutional Review Board approval and written informed consent that includes information about HPV serological testing;
- Data generated from the WHO HPV LabNet HPV serology proficiency studies will become the property of WHO, and may be analyzed for publication by the HPV LabNet either as an internal document or peer-reviewed manuscript;
- Donations will be duly acknowledged by the WHO and the HPV LabNet for the kind donation of these critical reference samples. The WHO HPV LabNet will provide data on all comparisons to donors if requested.

For further information about the donation and additional technical information, contact **Dr. Joakim Dillner** at joakim.dillner@med.lu.se. In order to proceed with this project in a timely manner, the WHO HPV LabNet would greatly appreciate responses from prospective donors by end of **January, 2009**.

3. Evaluation of HPV16 Serology - A Collaborative Study on Direct VLP-ELISA Conducted by HPV LabNet

This study was primarily intended as a preparation for an international collaborative study on HPV serology that will have the following goals:

- Serology established at all labs within the HPV LabNet;
 - Inter-lab variation determined - consistency of performance investigated;
 - International "cut-off" agreed (at present, each lab uses own cut-off);
 - A standardized assay established that will be described in an HPV Laboratory Manual and could be used for large scale serology in monitoring of vaccination.
- The study was based on a single source of HPV16 VLPs. Laboratories blindly tested previously characterized sera (six HPV16 seronegatives from virginal women, and eleven HPV16 DNA positive from cervix cancer patients), and the WHO reference reagent for HPV16 antibodies (Code: 05/134). Laboratories were asked to use the candidate SOP for the WHO HPV Laboratory Manual. All shipments were sent by Courier at ambient temperature. The practical use of the test appeared to have no major problems. All laboratories scored all positive sera as positive and the negative sera were mostly scored as negative.
 - In summary, the WHO HPV LabNet now has a SOP that has been used in reference laboratories around the globe and has performed satisfactorily. This study gave several indications for modifications in the SOP, in particular for clarifying ambiguities. All laboratories have shown that they can do HPV serology and a preliminary suggestion for an international consensus cut-off has been found. It seems highly likely that an international collaborative study that uses a standard, well characterised supply of VLPs and a larger validation panel of serum samples could result in establishment of a high performance, high reproducibility HPV serology method.

4. International Standards (IS) / Reagents for HPV Assays

Establishment of 1st WHO International Standards for HPV DNA

- At the WHO 59th ECBS meeting, candidate DNA Standards for HPV types 16 (NIBSC code 06/202) and 18 (NIBSC code 06/206) were established as IS, each with an assigned potency of 5×10^6 International Units (**IU**) per ampoule or 1×10^7 IU/mL when reconstituted in H₂O, as directed.
- Source material for the IS's was recombinant HPV plasmid DNA, encoding the full-length viral genome of HPV16 or HPV18.
- An international collaborative study of 19 laboratories (from 13 countries) demonstrated utility of these ISs in harmonizing assays for amplification and detection of HPV16 and HPV18 DNA.
- The first round of stability testing of these IS's has been completed at NIBSC:
 - Freeze-dried IS's were each stored at: 45°C, 37°C, 20°C, 4°C, -20°C and -70°C for 344 days.
 - In three independent analyses, IS's were reconstituted and tested concurrently in replicate by three different real-time quantitative PCR assays (HPV E6 and E7 type-specific PCRs and C33A human DNA PCR).
 - No drop in activity was observed for any of the assays for either IS after storage at temperatures up to 45°C for one year, thus demonstrating the stability of these ISs.

International Units OR Genome equivalents (GEq)?

- Some collaborative study participants and HPV experts have indicated that HPV DNA IS's should be assigned a unitage of GEq/ml rather than IU/ml, as they do not wish to be thinking IU for HPV16 and HPV18 and GEq for all other HPV types.
- At the 1st WHO HPV LabNet meeting in January 2008, it was proposed that a statement be included in the 'instructions for use' showing calculations and assumptions used to determine the theoretical GEq/ml and demonstrating that 1 IU is equivalent to 1 GEq for the candidate IS's.
- The definitive unitage of the 1st WHO IS's for HPV16 DNA and HPV18 DNA would therefore remain as IU while the traceability statement would allow users to equate IU with GEq.
- The 1st WHO IS's for HPV16 and HPV18 DNA are currently being relabeled at NIBSC and will be placed on the Institute's catalogue at <http://www.nibsc.ac.uk/products> in early **January 2009**.

Large-scale extraction, purification and freeze-drying of C33A DNA

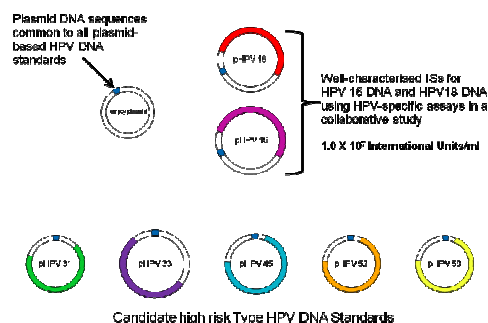
- For optimal calibration of HPV DNA assay diluents, inclusion of background human DNA is necessary to mimic clinical samples. NIBSC are currently investigating procedures for high-volume extraction and purification of human genomic **C33A DNA** for this purpose.
- To ensure optimal stability for international shipping at ambient temperatures, freeze-drying is being assessed:
 - DNA has been isolated from cells ($\sim 4 \times 10^{10}$) and formulated into 2.14 L, at a concentration of 65ng/ul C33A DNA in TE buffer and 5 mg/ml trehalose;
 - One ml aliquots have been prepared and freeze-dried;
 - Reconstitution into 1ml of H₂O would yield a concentration of ~ 40 x that of the C33A DNA used in the 1st IS's for HPV type 16 and 18 DNA;
 - The material could be used as a diluent (upon further dilution to a 1x concentration) in serial dilutions of IS in parallel with working standards for calibration of HPV DNA assays, with validation underway.

Proposal for HPV DNA high-risk type panel calibrated in International Units

- Establishment of HPV DNA IS's for HPV 16 and 18 was an important first step in harmonizing HPV DNA assays used in vaccine monitoring.
- However, a need for IS's for HPV DNA from additional high-risk types is necessary (as discussed at the 1st WHO LabNet meeting, in Geneva, January 2008).
 - HPV DNA standards for types 31, 33, 45, 52 and 58 were selected for development because accumulatively these types are responsible for ~17% of cervical cancers.
 - A proposal for the development of an HPV DNA panel for these high-risk types was endorsed by ECBS at its annual meeting in Geneva in October, 2008.
- The panel will consist of separate formulations of recombinant plasmids containing full-length genomic HPV DNA (sourced from the recombinant HPV plasmid preparations used in recent HPV LabNet genotyping proficiency studies) [Figure 1].
 - The formulation for 10⁷ HPV genome equivalents per ml (in a background of C33A DNA) and conditions for freeze-drying have been determined during the HPV 16/18 DNA studies.

Figure 1

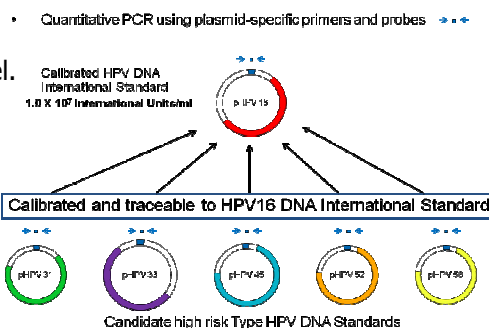
Schematic of plasmid-based HPV DNA standards



- ECBS has agreed to an alternative approach to the collaborative study, in efforts to reduce time and cost of calibration (in response to concerns expressed from participating in the collaborative study for establishing the HPV 16 and 18 DNA IS).
- Being plasmid-based HPV DNA standards, unitage of each candidate will be made by using the WHO IS for HPV16 DNA as a calibrator and qPCR method, thereby targeting a plasmid backbone sequence common to all members of the high-risk HPV DNA panel (i.e. traced to a single value assignment relative to an IS) [Figure 2].

Figure 2

Establishment of Unitage (Collaborative Study)



- NIBSC will design and validate the plasmid-based qPCR assay and provide primers and probes for use by study participants for assessing the HPV DNA high-risk type panel.
- HPV-specific assays (used at a smaller scale) will be used in HPV labs to validate the cloned HPV insert. Sequencing of the cloned HPV genome inserts may also be used for validation [Figure 3].

Figure 3

Validation of Cloned Insert



- Commercial and In-house quantitative and qualitative HPV NAT assays targeting various HPV sequences (L1, E6, E7, whole genome).
- The WHO HPV LabNet is currently conducting a genotyping proficiency study in which these plasmids are being tested. ⇒ Validation Data
- Sequencing of each genome insert ⇒ Sequence Validation

Dr. Dianna Wilkinson

*Division of Virology, National Institute for Biological Standards and Control (NIBSC)
Blanche Lane, South Mimms Hertfordshire
EN6 3QG, UK
dwilkinson@nibsc.ac.uk*

5. Congratulations – Professor Harald zur Hausen



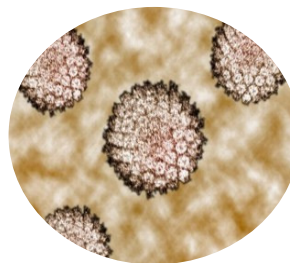
The WHO HPV LabNet Congratulate **Professor Harald zur Hausen.**

Co-recipient of the 2008 Nobel Prize in Physiology or Medicine:
for his vital role in the proposition and discovery that HPV
was the causal agent of carcinoma of the cervix.

Professor emeritus and former Chairman and Scientific Director,
German Cancer Research Centre, Heidelberg, Germany.

Prof zur Hausen, against the prevailing scientific view (in the 1970s), searched for HPV DNA within cancerous tumors, postulating that oncogenic HPV caused cervical cancer, the second most common cancer among women.

In a 10-year pursuit, in search of viral DNA (from oncogenic viral types) integrated into tumor cell genomes, he identified (in 1983) the novel **HPV16** within cervical cancer biopsies. By 1984, his laboratory had uncovered and cloned both HPV16 and 18, which are now consistently found in approximately 70% of cervical cancers worldwide.



The significance of the discovery of oncogenic HPV in cervical cancer, as well as HPV6 in genital warts, has provided immeasurable benefit to public health globally, given the widespread burden of this virus. Persistent HPV infection (the most common sexually transmitted infection) is responsible for around 5% of cancers worldwide. Approximately 40 HPV types are known to infect the genital tract – with around 15 posing a high-risk for cancer. Globally, more than ½ million new cases of cervix cancer occur annually, with HPV DNA detectable in around 99.7% of these cases.

In all, Prof zur Hausen's initial discovery of this heterogeneous family of viruses and characterization of their natural history of infection provided the foundation for understanding mechanisms of HPV-induced carcinogenesis and ultimately development of prophylactic vaccines. The quadrivalent vaccine has provided ≥95 % protection from disease caused by HPV16/18, as well as genital warts caused by HPV6/11. These vaccines, if implemented broadly across populations of young women (and possibly young men), should reduce the need for ablative therapy for precancerous lesions and overall burden of cervical cancer, especially given that many tests used in detecting early signs of cancer are not available in several resource poor countries.

For this invaluable research in HPV and
its potential to ultimately reduce cervical cancer,
we again warmly congratulate **Professor Harald zur Hausen.**

6. Recent Meeting Updates

WHO HPV Vaccine Advisory Committee (HVAC) Meeting

July 8th -10th, 2008, WHO Headquarters, Geneva, Switzerland

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

- Review new information critical to WHO policy on HPV vaccines;
- Prepare for a discussion about recommendations for HPV vaccine use in national immunization programmes at a Strategic Advisory Group of Experts (**SAGE**) meeting in November 2008;
- Provide advice about monitoring HPV vaccination programmes.

New clinical data reviewed:

- Quadrivalent vaccine (Gardasil) is safe and immunogenic in HIV-infected children in US;
- For both vaccines, long-term follow-up of phase II and III trials of adolescent females and women ≥ 25 years 5-6 year after vaccination demonstrate persistent antibody, clinical protection against CIN2+, and preliminary evidence of partial protection against oncogenic HPV types other than HPV 16 and 18;
- Continued follow up of trials and post-marketing surveillance indicate that both vaccines appear safe and do not cause adverse pregnancy, fetal, or neonatal outcomes;
- Co-administration of HPV vaccines with some common adolescent vaccines appears safe and does not impair immunogenicity to any antigen examined;
- Vaccination programmes can be cost-effective or cost-saving if vaccination costs (including vaccine price) are substantially reduced;
- Early experience in low, middle and high income countries shows that vaccines are generally acceptable and school-based delivery can achieve high coverage;
- Development and field testing of 2 rapid HPV tests that might be used to screen women in low and middle income countries is under way.

HPV vaccine policy developments reviewed:

- The WHO Categorization of Vaccine-Preventable Disease Project ranked prevention of cervical cancer as a "high priority" for countries;
- The Global Alliance for Vaccines and Immunization (**GAVI**) has decided to include HPV vaccines in its new investment strategy. Support may be possible if WHO recommends HPV vaccines for national immunization programmes, pre-qualifies HPV vaccines for UN procurement, and donor funding is secured;
- Regional meetings about HPV vaccine convened by July (in all regions except Africa) concluded that a comprehensive approach to cervical cancer prevention should include screening, treatment and vaccination. However, several factors pose barriers to vaccine introduction in many countries: current high vaccine prices, limited systems to routinely deliver vaccines to the target age group, and limited capacity to monitor the impact of vaccination programmes;
- WHO convened agencies that may be involved in future HPV vaccine funding or procurement for low and middle income countries middle and will promote continued interagency dialogue that may accelerate vaccine financing and supply. Manufacturers have pledged preferential pricing for public sector programmes.

Recommendation for monitoring HPV vaccination programmes:

- Unique monitoring approaches tiered to country income and capacity are needed, but a lack of monitoring systems should not delay vaccine introduction;
- All countries should monitor vaccine coverage and safety using existing passive systems as with all new vaccines;

- More complex population-based or sentinel monitoring activities should only be undertaken if sustainable and well-designed to avoid misleading conclusions: monitoring cancer and pre-cancers incidence and mortality through population-based cancer or cytology/histology registries; HPV-type distribution in cancers and pre-cancers; and prevalence of vaccine and non-vaccine HPV types;
- Population sampling strategies require careful design to allow international comparisons of impact. LabNet should focus on standardizing specimen collection and assays;
- WHO could support surveillance by developing clear objectives, standard data elements and surveillance guidelines for low and middle income countries;
- LabNet should initially focus on DNA testing because the serologic correlate of vaccine protection remains unknown;
- Stable, long-term funding is needed for LabNet.

Dr. Kathleen Irwin

*Initiative for Vaccine Research
World Health Organization,
GENEVA, SWITZERLAND
irwink@who.int*

2nd WHO HPV LabNet Meeting

November 17th - 19th, 2008, WHO Headquarters, Geneva, Switzerland

The WHO HPV LabNet convened, with several objectives:

- Evaluate and review data/results from recent HPV LabNet collaborative studies;
- Discuss further revision of a draft HPV Laboratory Manual;
- Review progress made in HPV LabNet and in each reference laboratory;
- Develop HPV LabNet work-plan for the next period;
- Share laboratory experience in countries in supporting HPV surveillance and vaccination impact monitoring.

The meeting was attended by HPV LabNet members, WHO Regional Offices and close partners (DKFZ, IARC).

The meeting was very productive with agreements on:

- Inclusion of one HPV genotyping protocol and one VLP-ELISA protocol (which have been evaluated by the HPV LabNet) in the HPV Laboratory Manual;
- Planned HPV LabNet collaborative studies on VLP-ELISA (phase 2) and a pre-study on HPV neutralization assay;
- HPV DNA genotyping proficiency study and confirmatory testing plans for 2009;
- Next steps with sourcing qualified VLPs, serum samples and developing ISs;
- Overall HPV LabNet work-plan for 2009.
- Due to the difficulties in sourcing critical materials, **WHO has opened calls for VLPs and serum samples for use in HPV LabNet studies.**

General recommendations were made to WHO:

- Long term sustainability of HPV LabNet should be pursued;
- International guidelines on HPV surveillance and vaccination monitoring need to be developed. This is essential for the HPV LabNet to develop infrastructure, testing formats, and testing capacity.

A meeting report is being developed and will be posted on WHO website.

7. Upcoming Meetings

25th International Papillomavirus Conference & Clinical Workshop

May 8th -14th, 2009, Malmö, Sweden. www.hpv2009.org

8. Useful Web Links

- <http://www.who.int/immunization/en/>
- <http://www.who.int/biologicals/en/>
- More information about WHO HPV LabNet:
http://www.who.int/biologicals/areas/vaccines/hpv_labnet/en/index.html
- <http://www.who.int/hpvcentre/en>
- <http://www.nibsc.ac.uk/products>
- <http://www.iarc.fr/>
- <http://www.ipvsoc.org/index.html>

9. Contributions for Next Edition of HPV LabNet Newsletter

The 4th WHO HPV LabNet Newsletter

Please forward suggested contributions within the next four months to the Co-Editors of the HPV LabNet Newsletter: suzanne.garland@thewomens.org.au and matthew.stevens@mcri.edu.au. Welcomed contributions include: local initiatives; pertinent projects; prevalence data for HPV DNA, especially genotype specific sero-surveillance; etc. Importantly, such contributions are sought from the wider global HPV community.

The HPV LabNet Newsletter is published 6-monthly in English by:
World Health Organization, Immunization, Vaccines and Biologicals, CH 1211 Geneva, Switzerland
Contact: Dr Tiequn Zhou, Scientist *Email: zhout@who.int*

Please address any correspondence to Co-Editors:
Prof. Suzanne Garland, *Email: suzanne.garland@thewomens.org.au*
Dr. Matthew Stevens, *Email: matthew.stevens@mcri.edu.au*
Department of Microbiology & Infectious Diseases,
The Royal Women's Hospital, Locked Bag 300, Parkville, Victoria 3052, Australia