



# WHO HPV LabNet - Newsletter 02



## 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 2nd edition of the 6-monthly newsletter.

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## 1. WHO HPV LabNet: 2008 Priorities

The two main priority areas of the HPV Laboratory Network (LabNet) in 2008 are Standardization of assays for HPV DNA and serology as well as Capacity Building.

### Standardization

- In collaboration with the WHO International Laboratory for Biological Standards, National Institute for Biological Standards and Control (**NIBSC**), contribute to the development of International Standards (**IS**) and reference reagents (e.g. HPV type 16 and 18 specific antisera and DNA panels, monoclonal antibody quality control panel for VLPs).
- Identify appropriate assays for HPV DNA and serology type-specific testing via proficiency studies and HPV LabNet inter-laboratory collaborative studies.

### Capacity Building

- Follow-up on the 1st HPV DNA proficiency study (Oct 07' - Jan 08').
- Conduct a 2nd proficiency study (for HPV DNA), in addition to a confirmatory testing and proficiency study for serology.
- Ensure all HPV LabNet Regional Laboratories are capable of performing both HPV DNA and serology testing.
- Sharing of information and/or knowledge on Quality Assurance (**QA**)/Quality Control (**QC**) within the LabNet. This will aid in developing a consensus "standardized/recognized" QA scheme for providing guidance to other laboratories.



## 2. Expert Opinions

### What Are We Measuring With HPV Serology Assays?

Most HPV serologic assays measure the ability of antibodies to bind to type-specific, conformational epitopes present on virus-like particles (VLPs) or their subunit capsomers. Each capsomer, composed of 5 L1 proteins, contains surfaced exposed loops to which antibodies bind. Most studies have used an ELISA format in which VLPs are either adhered directly to the plate, or a conformation-dependent monoclonal antibody (MAB) is bound to the plate and used to capture VLPs. A few studies have shown that capsomers can be used as antigen targets. Recently, a multiplex assay was developed with GST-L1 fusion proteins, which presumably assemble into capsomers, tethered to Luminex beads. Competition ELISAs or Luminex assays use a tagged form of a conformation-dependent MAB bound to the VLP and measure the ability of serum antibodies to displace the MAB. In characterizing the HPV antibodies that occur in natural infections, numerous studies have shown that the antibody responses are slow to develop, persist at least for years, are low titer, and are not detectable in 20% or more of individuals in whom HPV infections are measured by HPV DNA detection in the genital tract.



The above assays do not distinguish between neutralizing antibodies and antibodies that may simply bind the VLPs (Binding antibodies). To address this, an assay was developed using pseudoviruses, i.e. a VLP that contains both L1 and L2 and packages a plasmid expressing a reporter such as secreted alkaline phosphatase. The pseudoviruses are mixed with serum to determine if it contains antibodies that reduce the ability of the pseudovirus to infect an established cell line. The generation of a neutralizing antibody response is a likely correlate of protection against HPV infection. Studies have mapped the epitopes recognized by neutralizing MAbs to the surface loops and to the C-terminal arm of L1 that links the neighboring capsomers. Moreover, the neutralizing MAbs have been shown to block distinct steps of viral entry with ones that binds the loops (V5/E70) blocking attachment to the extracellular matrix and one that binds the arm (U4) blocking attachment to cellular glycosaminoglycans. Studies to more fully delineate the neutralizing antibodies in natural infection and following vaccination are ongoing.

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### Optimization of An HPV Virus-Like Particle (VLP) Based ELISA

Detection of IgG antibodies to HPV capsid (L1) proteins, or VLPs, by ELISA can identify type-specific, stable, lifetime exposure to HPV. Such serological assays have become important tools for epidemiological studies, and may prove as a useful adjunct to HPV DNA testing to identify women at higher risk of developing high-grade cervical neoplasia. In addition, serology will be a valuable commodity both pre- and post-vaccination against HPV infection, to determine prior HPV type-specific exposure, as well as assess antibody responses after vaccination.

Globally, such ELISA assays exhibit variation. The first WHO international collaborative study on the standardization of HPV antibody detection<sup>1</sup>, showed that use of a uniform internal standard (HPV16 antibody standard) and cut-off value, were the most important factors for improved inter-laboratory assay

<sup>1</sup> Ferguson M, et al. 2006. Results of the first WHO international collaborative study on the standardization of the detection of antibodies to human papillomaviruses. *Int J Cancer* **118**:1508-1514.

variation. Differing VLP qualities, serum dilutions, reagents and procedures may also be important for ELISA standardization.

### Methods of Optimization:

**VLP** - VLP quality is of utmost importance for reliable ELISA performance and can be ensured by:

- Electron microscopy assessment;
- Testing ELISA using type-specific monoclonal antibodies;
- Determining VLP type-specific reactivity as an antigenic unit and appropriate coating concentrations;
- Use of a heparin-based ELISA;
- Universal supplier of VLPs to resolve variation in VLP quality.



**Blocking solutions** - These can enhance the ELISA, reducing background and enhancing sensitivity and specificity.

**Cut-off-value** - Determination of a cut-off-value for ELISA positivity is crucial. The most common method is the use of mean OD values of negative serum plus 2-5 standard deviations of the mean.

**ELISA standardization and Quality Control** - To effectively monitor assay variations between: ELISA plates; reagent batches; day of test; and personnel, use of control sera on every plate is imperative (comprising of high, medium and low positive sera, and

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negative serum). Use of type-specific standard antibody facilitates agreement of ELISA results between laboratories by standardizing antibody levels into internationally comparable Units. Use of WHO Reference reagents will improve test performance, reproducibility and comparability.

A Reference Reagent for **anti-HPV16 antibodies** was formally approved by WHO in October 2007, and is now available from NIBSC. This material has an assigned unitage of 5 Units per ampoule (10U/mL reconstituted). Order forms and information are available at:  
<http://www.nibsc.ac.uk/products/faq.asp>

The primary focus of the WHO HPV LabNet for standardizing HPV antibody detection will be on developing IS and consensus of cut-off values.

## Monitoring and Evaluating HPV Vaccine Impact

Subsequent to implementation of HPV vaccination, an imperative "downstream" activity involves the monitoring and evaluation of vaccine impact. However, this requires consideration in these early stages post-vaccination, particularly process and impact indicators.

**Process indicators** - Need to be measured through routine national immunization programme surveillance.

**Vaccine impact** - The earliest indicator of impact will be seen through a reduction in infections caused by the vaccine-specific HPV types. Impact on early stage abnormalities (CINI/II) and/or genital warts (with quadrivalent vaccine "Gardasil") are likely to be seen much earlier than that on cervical cancer. Monitoring these outcomes in pilot studies will be useful for early assessment of vaccine impact and potential breakthrough cases. Data from such studies should be used to develop protocols for disease surveillance. Additionally,



systems for monitoring adverse events should endeavor to capture HIV status and pregnancies, since there is no data on the use of the vaccines in HIV-infected subjects and, while the data on pregnancy so far are reassuring, further monitoring is warranted.

The long-term impact of HPV vaccination on incidence of, or mortality from, cervical cancer and other HPV-related cancers will not be observed until at least 10 years, and more probably 30 years, after introduction. Where cancer registers exist, it will be important to include data on precancerous cervical lesions, which can be used as a proxy for invasive cancer for monitoring purposes. Where cancer registries do not exist or are of poor quality, countries are advised to monitor vaccine coverage and the outcomes of post-marketing surveillance in other countries. In the future, such surveillance may best be achieved using molecular markers for HPV infection, provided that appropriate tests are available and affordable. In particular, the reporting of adverse events will be important; this will require the cooperation of the private sector.

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### **3. Summary of WHO International Collaborative Studies and HPV LabNet Studies in 2007**

#### **Evaluation of International Standards [IS] for HPV16 and HPV18 DNA**

The need for establishing IS for HPV DNA testing has been discussed at various consultations with a feasibility study undertaken, demonstrating that IS would benefit assay standardization for HPV detection and quantification.

##### **International Standards (IS)**

Primary standard for any analyte, with an intended use of:

- Calibration of secondary "working" standards";
- Maintaining the unit continuity;
- Facilitating assay validation and assessment of sensitivity by test laboratories.

It is envisaged that the IS will be used by reagent manufacturers and/or organizations who will prepare working standards or run controls for use in their own and other laboratories.

Candidate IS for HPV16 and HPV18 DNA for use in nucleic acid amplification technology (NAT)-based assays were evaluated in a collaborative study of 19 laboratories (across 13 countries). These candidate ISs (from NIBSC) were freeze-dried preparations of HPV16 and HPV18 DNA.

A broad spectrum of commercial and in-house assays were used to assess IS prepared from bulk preparations of cloned plasmid containing full-length HPV16 or HPV18 genomic DNA. Data sets were returned from quantitative ( $n=15$ ) and qualitative ( $n=46$ ) assays.

There was no significant loss in potency for HPV18 DNA and a slight loss in potency for HPV16 DNA following freeze-drying; although this may not be scientifically relevant when assay precision is considered. Individual

laboratory mean estimates for each study sample were grouped around the theoretical HPV DNA concentrations of the reconstituted ampoule ( $1 \times 10^7$  HPV genome equivalents/ml). Inter-laboratory agreement was markedly improved when the potencies of the study samples were made relative to the candidate IS.

The data demonstrated the utility of these candidate ISs in harmonizing HPV16 and HPV18 DNA NAT-based assays.

#### **1st HPV LabNet Proficiency Study to Evaluate HPV DNA Typing**

A proficiency panel produced by the Global Reference Laboratory in Sweden, which was composed of 14 oncogenic HPV types and 2 benign HPV types, was evaluated and confirmed, then shipped to 10 other participating HPV LabNet laboratories, including NIBSC, for testing (**Table 1**).

Laboratories using the Roche HPV genotyping test reported very good results for all genotypes tested (except HPV39 and 68). Similar problems with HPV39 and 68 detection were observed using GP5+/6+ and PGMY PCR. The prevalence of these two oncogenic genotypes is comparatively low (such that this detection problem may be insignificant). However, it could also signify a systematic underestimation of these types with such assays, thereby misrepresenting their significance. Modified GP5+/6+ PCR and type-specific real-time PCR readily detected HPV39 and 68.

**Table 1. The 1st HPV LabNet proficiency study for HPV genotyping: number of labs, tests used, genotypes targeted and sample composition.**

HPV LabNet Labs	Different HPV genotyping tests used *	HPV types tested	Genome equivalents per 5ul	Single HPV types	Multiple HPV types	HPV negative
11	6	16		2	4	1
Malmö	<b>Commercial Assays</b>	6 45	500	16	16, 45, 52, 33	
Lausanne	HPV Linear Array	11 51		18	11, 18, 31, 51	
South Africa	(Roche Diagnostics)	16 52			35, 39, 59, 66	
CDC, USA		18 56			6, 56, 58, 68	
Japan	<b>In-house Assays</b>	31 58				
Australia	GP5+/6+ PCR	33 59	50	16	16, 45, 52, 33	
NIBSC	Modified GP5+/6+ PCR	35 66		18	11, 18, 31, 51	
Heidelberg	PGMY PCR	39 68			35, 39, 59, 66	
Thailand	RFLP				6, 56, 58, 68	
Tunisia	Type-specific RT-PCR		5	16		
India				18		

\* HPV test used does not correspond with the HPV LabNet Lab  
RFLP: Restriction fragment length polymorphism; RT-PCR: Real-time PCR

Technical improvement of these assays was deemed imperative to enable sensitive detection of HPV39 and 68. Laboratories who exhibited several false negative and/or false positive results, or whose method was deemed unsuitable for large-scale genotyping, were recommended to change their genotyping method. The LabNet has proposed to include an example of a robust method that works well (such as PGMY PCR with typing by reverse blotting), with detailed instructions on how to perform it in the WHO HPV Manual.

Other findings include instances of HPV types that were not within the proficiency panel, possibly reflecting aliquot contamination, or degradation due to transport or storage issues, resulting in cross-reactivity. The latter is currently being investigated. Laboratories using the Roche HPV genotyping test also have the dilemma on HPV52 reporting, due to a cross-reactive probe for HPV52 detection. HPV52 infections cannot be confirmed/excluded in the presence of genotypes 33, 35 and/or 58 (without a supplementary test).

#### 4. Projects Ongoing

- Promising assays for HPV DNA and serology testing are being evaluated for transferability by the WHO HPV LabNet and for the development of a WHO Global HPV Laboratory Manual;
- A 2<sup>nd</sup> WHO HPV LabNet Proficiency Study for evaluating HPV DNA typing methods to include all WHO HPV LabNet members and additional external laboratories who are interested. Both in-house assays and commercial assays will be evaluated. **Call for participation is available on WHO HPV LabNet website:**  
[http://www.who.int/biologicals/areas/vaccines/hpv\\_labnet/en/index.html](http://www.who.int/biologicals/areas/vaccines/hpv_labnet/en/index.html)

- Qualified VLPs are being sourced by the LabNet for the provision to the labs to use in HPV serology testing;
- Development of monoclonal antibody quality control panel for VLPs;
- Development of IS for anti-HPV type 18 sera.

## 5. Recent / Future Meetings

### **WHO HPV LabNet Meeting**

November 9th, 2007, Beijing, China

Meeting of the WHO HPV LabNet in which topics discussed included: WHO Manual for HPV Diagnosis;

- Uniformity in reporting the reporting of HPV testing and typing (data handling);
- Confirmatory HPV DNA testing (specimens with ambiguous HPV results to be sent to Global Reference Laboratory for confirmation);
- Results of a collaborative study on HPV16 and 18 DNA standards;
- Results of a HPV DNA proficiency panel;
- Progress of a HPV16 antibody standard;
- Programme for improved quality of HPV serology testing;
- HPV LabNet newsletter;
- Reporting of ongoing HPV surveillance studies;
- Joining the WHO HPV LabNet.

### **WHO Meeting on Standardization of HPV Assays and the Role of HPV LabNet in Supporting HPV Vaccine Introduction**

January 23rd – 25th, 2008, WHO Headquarters, Geneva, Switzerland

The aim of this meeting was to facilitate the progress of the LabNet and ensure its activities are in line with global expectations/demands. Some of the issues discussed included:

- Review of the current status in HPV testing areas towards standardization;
- Review of progress in the development of IS and potential needs for standardization;
- Review of current status and progress made by WHO global HPV LabNet and plan for the next;
- Discussion of role and function of HPV LabNet in supporting global HPV vaccine introduction;
- Review of the concepts and basic structure of a WHO HPV laboratory manual.

Overall outcomes of this meeting centered around the role of the HPV LabNet in supporting the introduction of HPV vaccines, specifically: HPV LabNet capacity building; development and standardization of appropriate assays to satisfy global needs; development of IS and promoting their use; and improved communication between WHO LabNet and public health authorities in different countries. The full meeting report will be made available on the WHO HPV LabNet website.

### **AOGIN** (at time of publication)

May 29th - 31st, 2008, Seoul, South Korea

### **WHO HPV Vaccine Advisory Committee Meeting**

(formerly known as the HPV Expert Advisory Group)

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

### **EUROGIN**

November 12th - 15th, 2008, Nice, France

## 2nd WHO HPV LabNet Meeting

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

## 6. Useful Web Links

- <http://www.who.int/immunization/en/>
- <http://www.who.int/biologicals/en/>
- [http://www.who.int/vaccine\\_research/diseases/hpv/labnet\\_call/en/index.html](http://www.who.int/vaccine_research/diseases/hpv/labnet_call/en/index.html)
- <http://www.who.int/hpvcentre/en>
- More information about WHO HPV LabNet:  
[http://www.who.int/biologicals/areas/vaccines/hpv\\_labnet/en/index.html](http://www.who.int/biologicals/areas/vaccines/hpv_labnet/en/index.html)

## 7. Newsletter Distribution

The WHO HPV LabNet website was officially launched on 12th December 2007. Efforts are being made to deliver the HPV LabNet information and 6-monthly Newsletter to a wide audience through LabNet dissemination, WHO Regional Offices etc; and thus far has listings on the International Papillomavirus (IPV) website (<http://www.ipvsoc.org/index.html>) and WHO website ([http://www.who.int/biologicals/areas/vaccines/hpv\\_labnet/en/index.html](http://www.who.int/biologicals/areas/vaccines/hpv_labnet/en/index.html)).

## Call for contributions for the 3rd WHO HPV LabNet Newsletter

Please forward suggested contributions within the next four months to the Co-Editors of the HPV LabNet Newsletter: [suzanne.garland@rch.org.au](mailto:suzanne.garland@rch.org.au) and [matthew.stevens@mcri.edu.au](mailto:matthew.stevens@mcri.edu.au). Welcomed contributions include: local initiatives; pertinent projects; prevalence data for HPV DNA, especially genotype specific sero-surveillance; etc.

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