Preface: This newsletter aims to provide a brief and updated overview of the WHO HPV LabNet activities, this being the 4th edition of the 6-monthly newsletter.

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2. Availability of International Standards and Reference Reagent  
3. Typical Activities of a HPV LabNet Regional Reference Laboratory - Thailand  
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10. An Update: Participating HPV LabNet Laboratories (May, 2009)

1. HPV LabNet Activities – NIBSC & Global Reference Laboratories

WHO/HQ convened the two HPV LabNet Global Reference Laboratories (GRL) (Malmo, Sweden and CDC, USA) and National Institute for Biological Standards and Control (NIBSC, UK) in March 2009 to review progress of current activities, discuss issues raised as well as plan the next.

**GRL – Sweden**

- **2nd HPV DNA Proficiency Study, 2008**
  A detailed summary report is being prepared for dissemination to all participating laboratories prior to publication. A list of potential commercial assays is being drawn due to strong recommendation by several labs for supporting HPV surveillance as a network.

- **3rd HPV DNA Proficiency Panel, 2009**
  Due to resource constraints, this HPV DNA proficiency study will be for HPV LabNet members only and likely to be initiated in August 2009. HPV 39 and 68 A and B constructs have been re-cloned, as suggested from the 2nd proficiency panel. Extraction controls preferred in the form of SiHa or HeLa cells, or C33A cells spiked with HPV plasmid DNA.

- **Donation of sera samples**
  Various LabNet laboratories donated sera for inclusion in the proficiency panel, yet were of insufficient volume for subsequent proficiency studies. **More serum samples are required.** Pooled samples are not appropriate.

- **Preparation for phase 2 VLP-ELISA collaborative study**
  This is likely to be delayed until September 2009. Availability of qualified VLPs and sufficient sera samples is essential.
GRL - CDC

• HPV Laboratory Manual
  The final reviewed version of the LabNet HPV Laboratory Manual will be ready for submission to WHO by June 2009. This is of top priority. This would be subject to the WHO IVB departmental clearance procedure, including final review and editing.

• Neutralization assay
  Results from the collaborative study were received from participating laboratories, with good agreement across all samples except one. It is hoped that a consensus standard operating procedure (SOP) will be reached for inclusion in the HPV Laboratory Manual. The developer of the generic protocol of the assay, Dr John Schiller, shall be duly acknowledged in the Manual.

NIBSC – UK

• International Standard of anti-HPV 16 serum
  Further data on the stability of 05/134, the anti-HPV 16 reference reagent, is now available. A full report detailing all additional stability studies undertaken to be submitted to ECBS, Oct 2009. Request to change the status of this material from WHO Reference Reagent (unitage in ‘units’) to International Standard (IS) (unitage in International Units, IU).

• Anti-HPV 18 serum – A top priority
  No offers of serum for use in preparing an IS had been forthcoming after calls for donation on the WHO Website. Screening of blood donations has commenced in the GRL, Sweden.

• IS of 5 High-Risk HPV types – Project is ongoing
  Project is ongoing. Once established as ISs, NIBSC will store and distribute to laboratories upon request.

• Monoclonal antibody panel for quality-controlled (QC) VLPs
  Aliquots of the antibodies have been sent to NIBSC and have been forwarded to the two GRLs. These have been tested in GRL, Sweden with appropriate working dilutions established, and thus available for use in the characterization of VLPs sent to NIBSC.

• VLP
  VLPs (mainly HPV 16 or 18 based) have been donated from several laboratories (both within and outside of the HPV LabNet) from various countries. VLPs to be evaluated by NIBSC by physicochemical methods, and by GRLs via serology assays (using pre-established SOPs). A second HPV LabNet serology study likely to commence in September 2009.

• Use of IS/RR
  Need to ensure the proper use of ISs across the HPV LabNet. The current Instruction For Use (IFU) does not include "calibration of secondary standards". Guidance to be included in the HPV Lab Manual; NIBSC to write SOP of how to use IS; Users to have more detailed information on the preparation and calibration of working standards; and NIBSC IFU to be included in the HPV Laboratory Manual.

• Purified human genomic DNA (C33A) for use as diluent of HPV DNA standards
  Potential alternative human cell lines to C33A DNA (negative for HPV DNA) are being considered due to C33A proprietary issues with ATCC. This may have a potential impact on the secondary standard-makers, particularly if C33A DNA is used for all ISs.
WHO

- **Gates project objectives - current priorities - expected "products/achievements"**
  A reminder of the aims and expected outcomes of the project funded by Gates.
  - Development of ISs for HPV DNA and serology: anti-HPV 16/18, HPV 16/18 DNA ISs.
  - Development of SOPs for HPV DNA assays (genotyping) and serology assays.
  - Development of HPV Laboratory Manual providing basic knowledge and assays for HPV serology, genotyping, QA/QC.
  - Capacity building in current HPV LabNet.
  - Providing a fundamental HPV LabNet.

This Bill and Melinda Gates foundation funded project will end in June 2010. Future plan and funding is under consideration at WHO.

2. **Availability of International Standards and Reference Reagent**

WHO International Standards (IS) for **HPV 16 DNA** (product number 06/202) and **HPV 18 DNA** (product number 06/206), as well the WHO Reference Reagent for **antibodies to HPV 16** (product code 05/134) are available from the National Institute for Biological Standards and Control (NIBSC).

To order, go to [www.nibsc.ac.uk/products/](http://www.nibsc.ac.uk/products/) and enter the product number into Keyword Search.

National Control Laboratories are exempt from the handling charges.

3. **Typical Activities of an HPV LabNet Regional Reference Laboratory: Thailand**

Each year, the HPV LabNet Regional Reference Laboratories (RRL) perform a number of tasks related to HPV detection and surveillance in and around their geographical location. Examples of such tasks are:

- Participation in HPV DNA confirmatory studies:
  - HPV DNA proficiency testing.
  - HPV 16 serology pre-study.
- Set-up and validation of the PGMY reverse blotting hybridization (described earlier) and GP reverse line blot hybridization (HPV LabNet studies).
- Providing of human serum samples for use in the development and international quality assurance of assays used in measuring HPV antibodies.
- Conducting epidemiological studies on HPV prevalence, type distribution and HPV 16/18 antibodies levels in women attending for routine cervical cancer screening at the National Cancer Institute, Bangkok, Thailand.
- Collaboration with ICO in studying HPV types in related cancers other than cervical cancer.

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4. Technology Transfer of a Non-commercial PCR Reverse Blotting HPV Genotyping Assay

At a WHO meeting on standardization of HPV assays and the role of HPV LabNet in supporting HPV vaccine introduction [January 2008], it was recommended that HPV LabNet should evaluate/validate an uniform in-house HPV genotyping assay, due to two major reasons:

- High variety of HPV genotyping assay formats;
- Expensiveness of commercial assays.

A uniform in-house HPV genotyping assay should be:

- Reliable; Reproducible;
- Cheaper; Transferable.

An in-house PCR reverse blotting hybridization (RBH) assay (PGMY-CHUV assay) developed at the RRL, Institute of Microbiology, CHUV, in Lausanne, was identified (from the 1st WHO HPV LabNet Proficiency Study for HPV DNA genotyping) as a potential candidate assay since it performed well and met the above specifications.

The assay is relatively cheap (3-4$ per sample, excluding DNA extraction) given the hybridization membrane with covalently linked probes can be reused ≥10 times (up to 400 samples).

Study was conducted in 2008 with the following main objectives:

- Evaluate a suggested standard operating procedure (SOP) for HPV typing in the LabNet.
- Improve the SOP following experience in the study.
- Identify any training needs.

4.1 Preparation and setup of the study

- Key reagents like primers and probes, positive control DNA and membranes were quality controlled (QC) at CHUV.
- Material was sent to the eight HPV LabNet participating laboratories.
- Participants were requested to perform the assay according to the SOP within three months of receipt of the material.
- During this time, CHUV provided help and troubleshooting to all laboratories.
- Each laboratory was requested to try the technique with the HPV proficiency panels and to give feedback on the assay and the SOP.

4.2 Results and discussion

Availability and cost of material

- All reagents used include common, readily available research products.
- Various reagents were cited as expensive by several laboratories – with the SOP subsequently modified to minimize their use. The low cost per sample can only be achieved if reusing the RBH membrane.

Technical issues

- Several laboratories underscored the higher hands-on time and steps of the RRL/CHUV assay compared to commercial assays. Whether this expected factual difference is a limiting factor in comparison to other in-house assays remains to be evaluated.
- Most laboratories could interpret correctly the SOP. Failure of one laboratory to perform the RBH could be explained by its not using the recommended detection reagent and not understanding another key step of the procedure.

Performance, quality controls, transferability of the technique and training needs

- Although the aim was not to assess assay performance, results of the proficiency panels did reveal a lack of sensitivity for detecting HPV 31, 33, 35 and 56 (especially among mixed
infections) across most laboratories. This was due to the buffer composition that favored clinical rather than analytical sensitivity. The SOP will be modified for epidemiological studies to favor highest sensitivity for all high-risk (HR) types;

- Contaminated probes at time of manufacture were identified through the CHUV quality control process, indicating that internal quality controls must be enforced for in house techniques. Future SOP will address this quality issue to provide guidance to laboratories and to oligonucleotide manufacturers.

- Four laboratories out of seven were able to perform the technique successfully, two labs were very close to reaching this goal with RBH background issues that would require little troubleshooting and practice, and one lab could not perform the technique as mentioned previously. For this laboratory, additional practical guidance by RRL/Switzerland would appear mandatory if it wishes to use this technique.

4.3 Conclusions

- Four of seven laboratories successfully used this relatively complex in-house assay, suggesting that technical transfer is possible.

- At 3-4$ per typing, this assay is much cheaper (10-20 times) compared to commercial assays. Provided sufficient technician’s time and expertise, such a non-commercial assay is certainly valuable in particular under financial constraints.

- Global implementation of HPV typing should be envisaged only under strict external and internal QC with the successful participation of the laboratories to proficiency panels and internationally-recognized quality control schemes. Compared to commercial assays where QC and standardization is centralized, the CHUV assay, like any other in-house assay, necessitates additional QC and performance monitoring to diminish the risk of inter-laboratory variability.

- Feedback from participating laboratories and our experience suggest that in-house assays are better adapted to R&D than to clinical laboratories reliant exclusively on commercial kits.

5. Establishment of an Indian HPV Vaccination Registry

- The Drug Controller General of India, the primary regulatory body for all clinical trials, has shown interest in the development of a National HPV Vaccination Registry.

- Assistance and advice is being sought from Australia, which already has a functioning HPV register. This includes sharing of the of the registry constitution, especially with respect to its various data entry fields and mode of operation.

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WHO HPV LabNet Regional Reference Laboratory Region: South-East Asia


This is the first WHO HPV DNA proficiency study open for participation to laboratories worldwide, following advertisement on the WHO website. 61 laboratories participated from the 6 WHO
Regions: Americas (16); Africa (1); Eastern Mediterranean (1); European (28); South-East Asia (2); Western Pacific (13).

The 1st HPV LabNet DNA proficiency panel targeted HPV 16 and 18; while this 2nd panel provided the possibility to analyze specificity and sensitivity of different typing assays to correctly identify 14 high-risk and 2 low-risk HPV types identified as the most important in HPV surveillance and monitoring.

**Study objectives were to:**
- Assess the proficiency of HPV typing assays when routinely used in laboratories worldwide;
- Evaluate sensitivity and type-specificity for HPV detection of the different HPV assays when routinely used in laboratories worldwide;
- Identify problems with any of the assays routinely used.

The proficiency panel comprised of 43 samples with purified plasmids diluted in a human placental DNA background. Types included: 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. The panel was distributed to 61 laboratories worldwide, with 85 datasets returned for analysis from 54 laboratories. >21 assay types were included.

Participating laboratories included: public health laboratories; research laboratories; diagnostic test manufacturers; and vaccine companies.

All WHO regions were represented in testing this panel, with the majority of laboratories from the Americas, European, and Western-Pacific regions.

**Results**
A total of 37 data sets results were obtained from use of commercially available tests. The most commonly used assay was the Linear Array HPV genotyping assay (Roche), which was used in 15 laboratories. Other widely used assays included the clinical array test CLART (Genomica) and INNO-LiPA (Innogenetics). 48 data sets were obtained using a variety of in-house assays.

<table>
<thead>
<tr>
<th>HPV assay type*</th>
<th>Number of data sets</th>
<th>HPV region targeted (primers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All assays</td>
<td>81*</td>
<td>L1/E1/E6/E7</td>
</tr>
<tr>
<td>Linear Array (Roche)</td>
<td>15</td>
<td>L1 (PGMY)</td>
</tr>
<tr>
<td>CLART (Genomica)</td>
<td>6</td>
<td>L1 (PGMY)</td>
</tr>
<tr>
<td>INNO-LiPA (Innogenetics)</td>
<td>6</td>
<td>L1 (SPF10)</td>
</tr>
<tr>
<td>PGMY-CHUV</td>
<td>7</td>
<td>L1 (PGMY)</td>
</tr>
<tr>
<td>In-house Type-specific PCR</td>
<td>7</td>
<td>L1 / E6 / E7</td>
</tr>
<tr>
<td>In-house 16/18 specific PCR</td>
<td>6</td>
<td>E6 / E7</td>
</tr>
<tr>
<td>DNA chip (Biocore)</td>
<td>4</td>
<td>L1</td>
</tr>
<tr>
<td>In-house Line blot</td>
<td>4</td>
<td>L1 (GP)</td>
</tr>
<tr>
<td>In-house PCR Luminex</td>
<td>4</td>
<td>L1 (GP or modified GP)</td>
</tr>
<tr>
<td>In-house PCR Luminex</td>
<td>4</td>
<td>E6 / E7</td>
</tr>
<tr>
<td>In-house Microarray</td>
<td>3</td>
<td>L1 / E7</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td>3</td>
<td>L1</td>
</tr>
<tr>
<td>Microarray (Genetel)</td>
<td>2</td>
<td>L1</td>
</tr>
<tr>
<td>DEIA LiPA assays</td>
<td>2</td>
<td>L1 (SPF 10)</td>
</tr>
<tr>
<td>In house PCR EIA</td>
<td>2</td>
<td>L1</td>
</tr>
<tr>
<td>PapilloCheck Microarray</td>
<td>1</td>
<td>E1</td>
</tr>
<tr>
<td>Type specific PCR (GenoID)</td>
<td>1</td>
<td>L1</td>
</tr>
<tr>
<td>In-house PCR Luminex</td>
<td>1</td>
<td>L1 (PGMY-GP)</td>
</tr>
<tr>
<td>PCR Luminex (Multimetrix)</td>
<td>1</td>
<td>L1 (GP)</td>
</tr>
<tr>
<td>PCR EIA (GenoID)</td>
<td>1</td>
<td>L1</td>
</tr>
<tr>
<td>In-house PCR sequencing</td>
<td>1</td>
<td>L1 (PGMY-GP)</td>
</tr>
</tbody>
</table>

*Assays used for HPV typing
- Four data sets were generated using assays that did not discriminate specific HPV types and are not included in the overall type-specific analyses presented here.
A data set was considered proficient when it detected at least 50 international units (IU) of HPV 16 and HPV 18 in 5ul and 500 genome equivalents (GE) in 5ul of the other HPV types, without having more than one false positive type detected, equaling a specificity of 97%.

- 19 (23 %) data sets were 100% proficient. The Linear Array and two different micro-array assays (Genetel and PapilloCheck) were the commercial tests with highest number of 100% proficient results.
- Several data sets generated by in-house assays based on type-specific PCR or by PCR with Luminex-based typing were 100% proficient.
- 28 data sets (33%) were classified as not proficient since all detected more than one false positive HPV type.

**Conclusions**

- The majority of laboratories demonstrated good performance in HPV DNA genotyping tests;
- HPV 16 and HPV 18 were the types detected at lowest IU in most data sets;
- Only 1 and 3 datasets, respectively, could not detect 500 IU / 5ul;
- HPV 52, 59 and 56 could not be detected in the 500 GE / 5ul amount by 25, 19 and 18 data sets respectively, suggesting that many surveys of circulating HPV types may underestimate the importance of these 3 types.
- This HPV DNA proficiency panel showed that it is possible to evaluate the sensitivity and specificity of different HPV typing assays, as well as the performance of participating laboratories.

**Use of such panels validating different HPV DNA tests allows for the standardization and quality improvement of HPV DNA typing results worldwide promoting improved comparability of data generated from laboratories worldwide.**

**Note:** Only HPV 16 and 18 were diluted to 5 IU (not all data sets analyze for all HPV types).
7. Recent Meeting Updates

25\textsuperscript{th} International Papillomavirus Conference & Clinical Workshop

May 8th -14th, 2009, Malmö, Sweden

The recent IPV meeting comprised of more than 2,000 registered participants.

The Conference/Workshop encompassed all aspects of Papillomavirus research, from clinical vaccinology to molecular biology.

More information can be found at: www.hpv2009.org

8. Useful Web Links

- [http://www.who.int/hpvcentre/en](http://www.who.int/hpvcentre/en)
- [http://www.nibsc.ac.uk/products](http://www.nibsc.ac.uk/products)
- [http://www.ipvsoc.org/index.html](http://www.ipvsoc.org/index.html)


The 5th WHO HPV LabNet Newsletter

Please forward suggested contributions within the next four months to the Co-Editors of the HPV LabNet Newsletter: susanne.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.

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10. An Update: Participating HPV LabNet Laboratories (May, 2009)

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