WHO HPV LabNet - Newsletter 07

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 7th edition of the 6-monthly newsletter.

Content: 1. Towards Standardized Extraction from Formalin-Fixed Paraffin-Embedded Tissue
2. Study Comparing Commercially Available HPV Genotyping Assays
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1. Towards Standardized Extraction from Formalin-Fixed Paraffin-Embedded Tissue

Archived tissues from diagnostic pathology laboratories are a valuable resource for determination of the type-specific HPV prevalence in cancer and pre-cancerous tissues prior to HPV vaccine introduction, as well as for monitoring the impact of HPV vaccination on type-distribution. Serial sectioning, with H&E histological review of first and last sections allows confirmation that intervening sections include the lesion of interest. While it is possible to extract DNA from these tissues, cross-links introduced by formalin fixation negatively impacts the yield and size of DNA fragments. Variations in fixation time associated with “routine” processing in histology laboratory further contribute to these difficulties. Determining a reliable standardized method for extraction of DNA from formalin-fixed paraffin-embedded (FFPE) is important if results from tissue archives are used to make public health decisions.

Several laboratories have placed protocols for DNA extraction from FFPE on the WHO HPV LabNet SharePoint, and have expressed interest in sharing tissues to allow direct inter-laboratory comparison of extraction and HPV typing results. Using tissue samples processed in a variety of clinical laboratories will strengthen the evaluation of extraction methods. Plans are still in development, but we envisage that each participating laboratory will be asked to:

- Contribute 5-6 tissue blocks for serial sectioning, ideally comprised of greater than 50% tumor, ~1 cm in diameter; selected to include 4-5 cervical or other HPV-associated tumors and one HPV-negative tumor (such as lymphoma or melanoma);
  - Unstained 10μm sections for HPV testing will be prepared in a central laboratory (or by submitting laboratories), with H&E confirmation of lesion every 25 sections.
- Use one protocol in common, as well as one other of their choice;
- Determine total DNA yield by quantitative PCR for a human gene as well as to report HPV detection and typing results.

HPV LabNet members will be contacted to determine their respective interest in this study within the next quarter. Participating laboratories will determine the final protocol and timeline for completion.

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2. Study Comparing Commercially Available HPV Genotyping Assays

Laboratories have met the technical challenge of HPV detection and typing by employing a variety of “home-brew” assays that have the advantage of low cost, yet require extensive training and investment of resources in reagent preparation and quality control (QC). The WHO HPV LabNet collaborated to have validated one such assay for inclusion in the WHO HPV Laboratory Manual. However, commercial kits have the advantage that manufacturers assume the burden of reagent QC and adapt the assay to achieve high-throughput and good inter-laboratory agreement. Thus, for laboratories initiating HPV genotyping, commercial kits require less hands-on investment in set-up and training. Commercial kits facilitate testing in large scale epidemiological studies and can contribute to stability of testing results over time and in different settings. Recommended by the experts in WHO meetings, the WHO HPV LabNet launched a study to compare current commercially available HPV genotyping assays.

Commercial kits were selected based on the anticipated needs of the HPV LabNet. The criteria were:

- Will detect and individually identify all or the majority of the thirteen high-risk (HR) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 as well as the two low-risk (LR) types 6 and 11 currently targeted by the quadrivalent vaccine;
- Do not require purchase of expensive instrumentation for assay performance and interpretation; and
- Have been used by participants in the WHO HPV proficiency testing program.

The kits selected are shown in the table below.

<table>
<thead>
<tr>
<th>Assay Name</th>
<th>Manufacturer</th>
<th>Principle</th>
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<tbody>
<tr>
<td>Linear Array HPV Genotyping Test (detects 37 types)</td>
<td>Roche Diagnostics</td>
<td>Reverse line blot</td>
</tr>
<tr>
<td>INNO-LiPA HPV Genotyping Extra (detects 28 types)</td>
<td>Innogenetics</td>
<td>Reverse line blot</td>
</tr>
<tr>
<td>RH Line Probe Assay (detects 18 types)</td>
<td>Qiagen</td>
<td>Reverse line blot</td>
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Performance will be evaluated based on sensitivity, specificity and reproducibility of results. Parameters of time and ease of use will be reported. Four sets of samples will be prepared:

1) Plasmid – titration: [8 samples in total]
   To evaluate competition between types with high and low copy numbers
   - A series of plasmids containing HPV16 or 18 genomic DNA in background of 25 ng human genomic DNA derived from the cell line HBT42 mixed to contain 50 genome equivalents (GE) of HPV18 in all 7 tubes and 10-fold increasing GEs of HPV16 from $5 \times 10^6$ to $5 \times 10^9$ copies. An additional sample will contain the human background DNA only and no HPV plasmid. Although $10^6$ copies might rarely be encountered under clinical testing conditions, it will serve to determine the fidelity limit of the assays.

2) Plasmids – detection individual types: [16 samples in total]
   To evaluate ability to detect 50 GE (or IU) of each type
   - Plasmids containing HR HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 and LR types HPV 6 and 11 will be prepared in background of human genomic DNA. Each sample is to contain $5 \times 10^4$ GE of one of the 15 types and 25 ng of genomic DNA. An additional sample will contain the human background DNA only and no HPV plasmid.
3) Plasmids – mix: [18 samples in total]
To evaluate sensitivity and specificity of detection with mixed types
- Plasmid DNA from the same 15 types will be mixed in samples containing two, three, four, five and six different types. Each will have a concentration of $5 \times 10^4$ GE with the additional genomic DNA. A total of 16 mixed type samples will be prepared so that each type is included three to four times in changing combinations.
- Plasmids with types 33, 35, 58 will not be mixed with type 52 to simplify interpretation of XR probe on the Linear Array.

4) Biological specimens – STM, FFPE: [62 samples in total]
To describe performance on extracts from epidemiological material (“real-life” performance)
- A random selection of excess anonymous samples from ongoing studies will be selected. Residual DNA extracted from cervical cells in specimen transport media (STM) and cervical cancer tissue from formalin-fixed paraffin-embedded (FFPE) blocks will be used. The FFPE tissues will have about a 90% probability to be HPV positive; the STM samples will have a high probability to contain multiple HPV types.
- 30 STM samples and 30 FFPE samples, 2 extraction controls

Samples will be prepared by personnel not involved in testing. Duplicate coded aliquots of each sample for each of the three tests will be prepared. Two technologists will independently perform testing using all three test platforms and independently record and interpret results. Testing will not be initiated until both technologists have verified competence with all three assays.

The sample series with HPV genomes in plasmid, single type and mixed types will allow an assessment of the assay sensitivity and specificity with regards to the detection threshold of 50 GE (or IU) per sample. They will also allow an evaluation of a test’s ability to detect multiple types and handle template competition. Since the template amount and genomic identity is controlled it will serve as a “gold standard” to evaluate these parameters.

Extracts from cervical cells with CIN3 or FFPE cervical cancer tissues will be utilized to compare performance on epidemiologic samples. The analysis for this test sample set will be descriptive and relative between the assays. Since some assay dependent variations of HPV typing results may always occur, a gold standard will not be available for absolute type determination in this sample set. The performance in actual samples will complement information with regards to the test’s performance in the plasmid sample series. Additionally, the duplicate testing will also allow evaluation of each assay’s reproducibility. Attention will also be given to the ease of use, technical requirements and adaptability of the assay set-up to different specimen types.

The study design was developed by WHO and both GRLs in the HPV LabNet. Study was initiated in October 2010 at the GRL/CDC, with anticipated completion by January 2011. Results will be reported by the study group.

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3. An Update: HPV LabNet International Collaborative Studies

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

In June 2010, the National Institute Biological Standards and Control (NIBSC) filled and freeze-dried pooled sera from women naturally infected with HPV 18 (*described in the 6th edition of this newsletter*). An international collaborative study is currently in motion, to assess the suitability of this freeze-dried serum to serve as the 1st International Standard (IS) for antibodies to HPV 18 for use in the calibration and standardisation of enzyme immunoassays and neutralization assays. WHO HPV LabNet members and other HPV laboratories that have established neutralization assays and/or immunoassays were invited to participate.

**Objectives:**
The aims of this collaborative study are to:

- Assess the suitability of the freeze-dried serum to serve as the 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 in a range of typical assays performed in different laboratories;
- Determine commutability i.e. to establish the extent to which the reference standard is suitable to serve as a standard for the variety of different samples being assayed.

This study is funded in part by WHO via a project funded by the Bill and Melinda Gates Foundation. Full details of the collaborative study were given in the study protocol; a draft of which has been provided to prospective participants. In brief:

- Each participant is requested to perform 3 independent assays on serially diluted candidate standard and additional coded samples using the enzyme immunoassay and/or neutralization assay for antibodies to HPV 18 routinely used in their laboratory;
- Confidentiality of each laboratory will be ensured with each participant being anonymous to the other laboratories;
- Assay data will be analysed at NIBSC by an experienced biometrician using standard statistical techniques, with a draft report to be sent to participants for comment prior to consideration by the WHO Expert Committee on Biological Standardization (ECBS);
- The finalized report will then be submitted to the WHO ECBS who will decide on the suitability of the candidate standard to serve as the IS.

The collaborative study will begin shortly after NIBSC receives all study materials from donors under Material Transfer Agreement. It is anticipated that dispatch of the study materials to participants will take place in the 4th quarter of 2010 or 1st quarter of 2011.

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The 4th WHO LabNet HPV DNA Proficiency Panel, 2010

The 4th WHO HPV DNA proficiency panel, produced by GRL/Sweden of WHO HPV LabNet and distributed by Equalis, was issued to worldwide laboratories including WHO HPV LabNet members and external laboratories upon their requests following an open call for quality assessment for HPV DNA testing. As with previous panels, the possibility to assess specificity and sensitivity for varied HPV typing assays, in terms of correct identification of 14 high-risk and 2 low-risk HPV types was provided.

Objectives:
- Assess the proficiency of HPV typing methodologies commonly used throughout HPV testing laboratories;
- Facilitate a comparison between assays and laboratories;
- Identify potential problems with such routine assays.

Methods:
- The 4th HPV DNA proficiency panel comprised of 43 samples, with single and multiple HPV types per sample, which were all purified plasmids diluted in a background of human placenta DNA.
- 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 (two different subtypes of HPV 68).
- Three additional samples: A, B and C, were cell lines used as DNA extraction controls.
- The panel was distributed to 105 laboratories throughout six WHO regions during June and August, 2010. There was no charge for laboratories to participate, with all shipments paid by the WHO HPV LabNet.
- Number (n) of participating laboratories in the WHO regions were:
  - African Region (n = 1)
  - Region of the Americas (n = 23)
  - European Region (n = 49)
  - South-East Asia Region (n = 9)
  - Eastern Mediterranean Region (n = 5)
  - Western Pacific Region (n = 18)

Results:
- 131 datasets with results were returned prior to the deadline from 98 laboratories (93%):
  - 74 laboratories submitted a dataset from one test only;
  - 16 laboratories submitted datasets from 2 different tests;
  - 7 laboratories submitted datasets from 3 tests; and
  - 1 laboratory submitted datasets from 4 different tests.
- Preliminary analysis of the data submitted shows that:
  - 72 of the datasets were generated using commercial assays
    - Linear Array was the most common assay used by 17 laboratories;
    - INNO-LiPA followed, used by 12 laboratories.
- 7 datasets correctly identified all HPV types in all 43 samples
- 28 datasets were 100% proficient for the HPV types detectable by the assay(s) used
  - A total of 35 datasets were 100% proficient (26%).
- 45 datasets were proficient for HPV typing, but not for all types detected by the assay performed.
- 51 (39%) of the datasets had more than one false positive result, they are not proficient according to the criteria in the WHO HPV LabNet proficiency study.

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The 4th WHO LabNet HPV DNA Proficiency Panel: Re-cloning of HPV types

The WHO HPV DNA proficiency panel, produced by GRL/Sweden, has been distributed annually since 2008. The panel is used to assess specificity and sensitivity for 14 HR HPV types and 2 LR types. Accurate and comparable HPV detection and typing methodology is essential and we continuously aim to improve the HPV proficiency panel in order to make the content suitable for evaluation of a large set of different HPV detection methods used by laboratories.

Since the panel contains cloned HPV-types, some detection systems may fail if the vector is positioned between the targets for the used primers (Figure 1). Indeed, PCR with L1 as the target gene initially failed in the identification of HPV 11, 39 and 58 using primers MY09/11 or PGMY09/11. Therefore, we re-cloned these HPV types, as well as HPV 51, in order to move the vector to a region of the HPV-genome that is less likely to be targeted for detection. By moving the vector to another position of the HPV genome the re-cloned HPV types were demonstrated to be detectable by PCR using the MY09/11 primers (data not shown) and were included in the 4th panel in 2010.

Activities in the pipeline:
A detection system with E1 as the target gene (PapilloCheck, Greiner Bio-One) can not detect cloned HPV 18. Since HPV 18 was originally cloned in the E1 gene at position 2441 (targeted by this method) we are now in the process to move the vector to another region of the HPV 18 genome. Furthermore, we also plan to include the entire genome of HPV 68a to the proficiency panel.

Acknowledgements:
The task of setting up a proficiency panel for use in the WHO HPV LabNet proficiency study was generously supported by HPV-researchers around the world and we would therefore take the opportunity to acknowledge Professors Ethel-Michele de Villiers (HPV 6, 11, 16 and 18), Gérard Orth (HPV 33, 39, 66 and 68a), Attila Lörincz (HPV 31, 35, 56), Wayne Lancaster (HPV 52), Keerti Shah (HPV 45), Saul Silverstein (HPV 51), Toshihiko Matsukura (HPV 58 and 59), Elisabeth Schwarz (HPV 68b) for giving their kind permission to use the cloned HPV types for the proficiency panel.

The L1 gene of HPV is the target for several commercially available screening tests, such as Roche HPV Amplicor™ that amplify a region of about 165 bp, using a mix of 12 HPV primers between the MY11 and the GP6+ primers, for simultaneous detection of 13 HR HPV types. The Linear Array test (Roche) use PGMY09/11 to generate amplicons for typing of 37 HPV types. The Abbott RealTime HR HPV screening test amplify about 150 bp using the GP5+/6+ primer mix consisting of three forward primers and two reverse primers, for detection of 14 HR HPV genotypes simultaneously. The SPF10 primers amplify about 65 bp that can be typed for 28 HPV types using the INNO-LIPA HPV Genotyping Extra kit (Innogenetics, Belgium). The E1 gene is the target for the PapilloCheck® HPV-screening test (Greiner Bio-One, Germany) which is a PCR-based DNA microarray system for the detection and identification of 24 HPV genotypes.
4. HPV LabNet Regional Reference Laboratory Activities: A Snapshot

HPV LabNet Regional Reference Laboratories (RRLs) actively participate in a number of tasks related to the detection and surveillance of HPV in/around their geographical location. Activities as such (over the past 6-month period) include:

**RRL, WHO Western Pacific Region**

The National Institute of Infectious Diseases (NIID), Japan aims at performing following functions:

- Basic and applied research on infectious diseases;
- Reference services for infectious diseases;
- Surveillance of infectious diseases;
- National control of biological products including vaccines.

Based on the financial support from the Japanese Ministry of Health, Labour and Welfare, our lab has been carrying out the HPV-related activities as RRL. Since one of the HPV vaccines (Cervarix™) was approved in Japan in 2009, we are preparing a new surveillance program for HPV type distribution in cervical cancer patients in Japan using the typing methodology validated in the WHO HPV LabNet HPV DNA proficiency studies.

Our HPV-related activities over the past 6 months are as follows:

**HPV prevalence survey**

- HPV genotyping of cervical DNA samples from 393 outpatients that had visited the NTT Medical Center, Tokyo showed that 48% were HPV-negative and 50% were HPV-positive, 31% of which corresponded to multiple infections. Percentage of HPV positives increased with the severity of lesions: normal cytology, 31%; low-grade squamous intraepithelial lesions (LSIL), 66%; and high-grade squamous intraepithelial lesions (HSIL), 95%. The top three frequently detected types were HPV 52, 16 and 58.

**Development of a novel high-throughput HPV typing system**

- The VeraCode-allele specific primer extension system on the Illumina BeadXpress platform was evaluated for its suitability as a method to detect and genotype HPV DNA. By using this system, sixteen clinically important HPV types (HPV 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) were correctly genotyped in a multiplex format.

**HPV DNA proficiency panel, 2010**

- We participated in the 4th WHO HPV LabNet DNA genotyping proficiency panel study using the PGMY-Reverse Blotting Hybridization (RBH) assay that was introduced from the RRL/Switzerland and described in the WHO HPV Laboratory Manual (in press). Result is being analyzed at GRL, Sweden.

**HPV research studies**

- We have found that anti-HPV 16 L2 neutralization antibody inhibits nuclear entry of HPV 16 pseudovirus-packaged DNA (Ishii, Y. et al. Virology. 2010, 406:181-8).
- We have reported a rolling-circle mode of HPV DNA replication in epithelial cell extracts, which may have physiological implications for HPV DNA integration into host cell chromosomes (Kusumoto-Matsuo, R. et al. Genes to Cells, in press).
During the past 6-month period, the Department of Microbiology & Infectious Diseases, located at the Royal Women's Hospital (RWH), Melbourne, Australia has performed a number of HPV-related activities.

Our Department, within the RWH, has been involved in:
• Both HPV diagnostic testing as well as varied research studies;
• Ongoing HPV surveillance.

**Current research activities**

- Continuation of Australian HPV genotype prevalence analysis (post-government funded HPV vaccination programme);
- Recent commencement of evaluation of the impact of HPV vaccination (school-based program) with regards to circulating HPV genotypes in this age group;
- Investigation of other HPV-related cancers – e.g. HPV genotyping of anal, oral, laryngeal and vulval cancers:
  - Investigation of HPV among anal cancers
    - HPV genotyping from FFPE specimens;
    - Study for the prevention of anal cancer – a longitudinal study of the epidemiology of HPV infection and related anal squamous intra-epithelial lesions in both HIV-/HIV+ MSM (≥35 years).
  - Oral HPV study (best transport mediums for self-collected oral samples);
- Finalizing/analysis of data from WHINURS study – an Australian-wide HPV genotyping prevalence study (pre-vaccination) to assess HPV-type variation between: Indigenous and non-Indigenous women, and remote/non-remote area of residence;
- Other studies – HPV variant analyses among different HPV-associated diseases (early-onset cervical cancers, vulval cancers).

**HPV LabNet activities (studies / meetings / communication):**

- Participation in HPV LabNet proficiency studies (4th HPV DNA recently completed);
- Involvement in QA panel testing and preparation, in collaboration with the Royal College of Pathologists of Australasia (RCPA);
- Communication and liaising with national/international laboratories for HPV prevalence studies among cervical cancers:
  - Sri Lanka, Papua New Guinea and Fiji;
- Cervical Cancer Not Yet Beaten (CCNYB) annual meeting (Oct 14th, 2010)
  - Discussion of varied HPV-related topics (inc. focusing on other HPV-related cancers)
- Preparation of twice yearly WHO HPV LabNet Newsletter;
- Recent acceptance of study describing evaluation of HPV type persistence following surgical intervention for high-grade disease *(Obstet. and Gynaeco., in press)*.

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**Brief Update on HPV Surveillance in USA**

HPV activities at CDC involve at least 4 different centers:

- National Center for Immunizations and Respiratory Diseases (NCIRD);
- National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention (NCHHSTP);
- National Center for Chronic Disease Prevention and Health Promotion (NCCDPHP), and
- National Center for Emerging and Zoonotic Infectious Diseases (NCEZID).

Activities and communication are coordinated through a CDC HPV Workgroup. The HPV laboratory, currently serving as one of the WHO HPV LabNet GRLs, is located in **NCEZID**. The laboratory is CLIA certified and performs HPV serology and HPV DNA detection and typing assays as part of several ongoing studies of HPV:

**Monitoring Cancer Disease Burden**

- Type-specific HPV prevalence in HPV-related cancers pre-vaccine (2004-5) (NCCDPHP)
  - CDC’s National Program of Cancer Registries (NPCR) and NCI’s Surveillance Epidemiology and Endpoint Results (SEER)-cover US;
  - Population-based sample of cervical, vaginal, vulva, anal, penile and oropharyngeal cancers in 4 states;
  - HPV detection and typing in FFPE blocks, virtual slide repository.

**Monitoring Pre-Cancer Disease Burden**

- CIN 2/3 in Emerging Infections Program (NCHHSTP)
  - Pilot with 4 sites to monitor CIN 2/3 diagnoses in defined catchment areas to allow denominator of screened women to be determined;
  - Subset CIN 2/3 investigated in detail (determine vaccination status);
  - Subset of histology samples cut for PCR with H&E of initial and final section;
  - Central histology review using web-based access of virtual slides;
  - Type-specific detection of HPV.

**Monitoring HPV Prevalence**

- National Health and Nutrition Evaluation Survey (NHANES)(NCHHSTP)
  - Population-based survey of US
    - Seroprevalence HPV 16; HPV 6, 11, 16, 18;
    - Type-specific prevalence in self-collected vaginal swabs initiated in 2002.

- VSD sentinel survey (NCHHSTP)
  - HPV detection in residual cervical samples collected prior to vaccine implementation from random sample of women 11-29 years
    - 2 sites, 6000 samples from each;
    - SurePath or STM collection media.

**Monitoring Vaccine Impact on Behavior and Health Care**

- Pilot study of “DNA Pap” in Breast and Cervical Cancer Screening Program.

**Translational Research**

- Sampling, high throughput testing;
- Dosing interval studies (with NIAID and NCHHSTP);
- Molecular markers of cervical neoplasia to improve cervical cancer screening;
- Interagency Agreement with NCI – Early Detection Research Network.
6. Meeting Reports/Updates

**WHO HPV Surveillance and Monitoring Meeting**
November 16th - 17th, 2009, WHO/HQ, Geneva, Switzerland

Since HPV vaccine is recommended for an age group that has not been routinely served by the Expanded Programme on Immunization (EPI), and since the population impact of the vaccine on cervical cancer cannot be measured until years after the vaccine has been introduced, it is generally recognized that new approaches to monitoring coverage and impact are needed for HPV vaccine. HPV vaccine impact monitoring involves engaging partners in the fields of immunization, sexually transmitted infections, reproductive health, adolescent health, and cancer.

Readers are encouraged to look at the summary report published on the WHO Weekly Epidemiological Record (WER), whilst those requiring more comprehensive details should read the detailed meeting report published by WHO (the first link below).

**Publication of “Report of the meeting on HPV Vaccine Coverage and Impact Monitoring” can be found on the following links:**
- [http://whqlibdoc.who.int/hq/2010/WHO_IVB_10.05_eng.pdf](http://whqlibdoc.who.int/hq/2010/WHO_IVB_10.05_eng.pdf)
- A summary report was also published (mid-2010) in the WHO WER -

**Key points:** Monitoring HPV disease is not a prerequisite to initiating an HPV vaccination programme nor is it an essential requirement of a programme. Monitoring the impact of HPV vaccine is complex and should be done with good technical support and a clear understanding of the caveats to avoid drawing erroneous conclusions. All countries should consider establishing or improving reporting to cervical cancer registries since such registries are necessary to measure the impact both of HPV vaccine programmes and cervical cancer screening programmes. Monitoring the prevalence of infection by HPV genotype among sexually active young women may provide an early indication of vaccine effectiveness but requires considerable commitment of resources for at least 5-10 years and good technical support so this strategy is not recommended for all countries.

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**WHO HPV LabNet Symposium: Laboratory testing in HPV vaccinology and HPV surveillance.**
**July 5th, 2010, 26th International Papillomavirus Conference, Montreal, Canada**

**Preface:** The symposium was organized by the two LabNet GRLs. The main aims were to review:
- Activities of the LabNet at GRLs and RRLs;
- Activities of official National HPV Reference Laboratories (NRLs) appointed by their respective countries in order to explore areas for collaboration and synergies with the GRLs and RRLs of the WHO HPV LabNet.

As it was an informal meeting organized without budget, only reference laboratories that were known to be attending the 26th International Papillomavirus Conference were invited to give presentations. **The Symposium had 10 speakers and about 60 attendees:**
Joakim Dillner (GRL, Sweden): Introduction and WHO HPV LabNet overview.
  • Updated on the standardization and different types of standards available: written standards (as e.g. found in the WHO HPV Laboratory Manual) and physical standards for measurement (as the IS for HPV16 Antibodies, HPV16 DNA and HPV18 DNA).
  • Ongoing projects in LabNet in collaboration with NIBSC:
    o IS for HPV 18 antibodies, a collaborative study is planned.
    o IS for DNA of HPV types 31/33/45/52/58 as well as 6/11.

Elizabeth Unger (GRL, USA): Activities of the GRL USA and HPV surveillance in the USA CDC’s approach to cross-cutting nature of HPV is to coordinate work through the 4 different centers addressing, immunization, STDs, cancer and laboratory.
  • Projects established to monitoring disease burden of cancer through national system of cancer registries and pilot to determine HPV prevalence in cancers. Pilot project to establish method to monitor incident precancer and type-specific prevalence. HPV type specific prevalence determined in national sample using self-collected vaginal swabs as well as national seroprevalence (males and females).

Carina Eklund (GRL, Sweden): WHO HPV LabNet Proficiency studies on HPV DNA genotyping and HPV serology.
  • Presentation of results from the 2009 HPV DNA proficiency study. 5 laboratories were 100 % proficient, 3 laboratories not proficient because of >1 false positive results.
  • 2010 HPV DNA proficiency panel open for worldwide participation. Distributed to 98 laboratories worldwide in the end of June 2010.
  • Results of the 1st WHO HPV 16 serology proficiency study. A proposed cut-off was established: mean + 3 standard deviations of negative control sera. 6 laboratories out of 10 were proficient in testing with > 50% sensitivity and 100% specificity.

Isabelle Heard (NRL, France): A national program for HPV genotyping in France.
  • The NRL has been created in November 2008 and is located at the Pasteur Institute. The laboratory performs genotyping in the National Cervical Cancer Screening Program. 6000 samples from different pathology laboratories are sent for HPV typing using Papillocheck at the NRL. Results from the study are expected by the end of 2012. This will be the baseline HPV type distribution before vaccination in France.
  • The NRL has developed a national QC program.
  • France has HPV vaccination program for girls of 14 years of age, but with low coverage. A low vaccine coverage may increase the risk for emergence of variants or/and new genotypes, underlining the importance of monitoring.

Heather Cubie (NRL, Scotland): Development and Operation of an HPV Quality Assurance (QA) program in Scotland.
  • Described the steps taken for Quality Assessment and QA that covers the entire testing program as well as the QCs used for each individual test.
  • The NRL has, in collaboration with the equalization and standardization agency UK-NEQAS, developed a QA program for HPV that started in 2005 with a pilot study on HPV typing of LBC samples. The pilot QC program included 12 clinical LBC specimens that were distributed to different laboratories for testing.

Anna-Lise Williamson (RRL, South Africa): HPV QA and surveillance in South Africa.
  • HIV is a major problem in South Africa with 5.5 million infected individuals, 900 000 of these are now on ARV treatment. Women on ARV treatment live long enough to develop cervical cancer. Genital warts are another problem for these patients.
• South Africa has no HPV vaccination program in place. The RRL is conducting several research projects to study both HPV DNA and sero-prevalence. Data from a study of couples were presented.
• A new molecular epidemiology laboratory will be established where the HPV testing will be performed by the RRL.

Herbert Pfister (NRL, Germany): Aims and duties of the German National Reference Center for Papilloma and Polyoma viruses.
• Germany has a system with government-appointed and funded reference laboratories for infections of public health relevance. As of today, only 18 infections have been appointed with NRLs. The fact that HPV is one of them shows the high priority given to HPV.
• The NRL works on improvement of diagnostic methods for HPV infection (DNA typing). A new assay for HPV typing in the Beta-papillomavirus group has been developed (Nature Protocols 5:1-13, 2010)
• Evaluation of new commercial test systems is performed. E.g. Nuclisens Easy Q HPV that detects E6/E7 mRNA for HPV 16, 18, 31, 33 and 45 has been tested.
• The laboratory is involved in the evaluation of the HPV vaccine introduction in Germany.

Maria Alejandra Picconi (RRL, Argentina): Activities of the Regional Reference Laboratory for the Americas.
• The RRL has established two methods for HPV DNA typing, the PGMY-RBH (CHUV) assay and a GP5+/6+ based line blot assay.
• The main focus of the laboratory is capacity building. A workshop with 20 participants from 13 countries in Latin America that included training on the HPV DNA typing methods as well as QC procedures was arranged during the spring 2010.

Simon Beddows (NRL, England): HPV vaccine surveillance and associated R&D.
• The NRL works to establish type-specific HPV prevalence in England before and after HPV vaccination (Howell-Jones et al., Br. J. Cancer 103:209-16).
• A study to evaluate the use of urine samples for HPV DNA typing in comparison with cytology (females) and penile swabs (males) was described.
• Two DNA typing methods are used, Linear Array and a Luminex-based assay, and serological capacity is being developed.

Christine Jonassen (NRL, Norway): HPV vaccine surveillance in pre-screening age cohorts in Norway: HPV testing in urine samples.
• Norway has a school-based vaccination program of girls 11-12 years of age. The screening program starts at age 25-29; there is thus a gap of more than 10 years when the effect of the vaccination cannot be studied.
• Plans to use urine samples for HPV testing and typing by MGP-Luminex. 400 positive samples are used for a validation study of the methodologies including extraction of DNA from urine. The surveillance program is planned to start in 2010.

Dr. Alberto Severini of the National Microbiology Laboratory, Public Health Agency of Canada and Dr. Mario Poljak of Institute of Microbiology and Immunology. Medical Faculty, University of Ljubljana, Slovenia informed the participants that also Canada and Slovenia have officially appointed NRLs for HPV, that perform similar activities.

The meeting was concluded with the statement that we had identified opportunities for intensified collaboration between HPV reference laboratories.
7. Recent Publications of WHO HPV LabNet work


The WHO HPV Laboratory Manual has gone through WHO approval. It is envisaged that this manual will be published soon.

8. Useful Web Links

- [http://www.who.int/hpvcentre/en](http://www.who.int/hpvcentre/en)
- [http://www.who.int/immunization/en](http://www.who.int/immunization/en)
- [http://www.who.int/biologicals/en](http://www.who.int/biologicals/en)
- [http://www.ipvsoc.org/index.html](http://www.ipvsoc.org/index.html)
- [http://www.iarc.fr](http://www.iarc.fr)
- [http://www.uicc.org](http://www.uicc.org)


Please forward suggested contributions within the next four months to the editor 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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