Preface: This newsletter aims to provide a brief and updated overview of the WHO HPV LabNet activities, this being the 5th edition of the 6-monthly newsletter.

Content: 1. WHO HPV Laboratory Manual: Purpose and target audience  
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1. WHO HPV Laboratory Manual: Purpose and target audience

This manual was developed by the WHO HPV LabNet based on knowledge and experience gained through its international collaborative studies over the past several years. This manual is in its final stages of preparation [led by Global Reference Laboratory (GRL)/CDC, US] and aims to be made available to the public by 2010. The objective of this manual is to assist in establishing good laboratory support required for implementation and monitoring of HPV vaccination programmes by:

- Providing a brief summary of the biology and natural history of HPV and the world-wide burden of HPV-associated disease;
- Discussing the role of laboratories and the WHO HPV LabNet in supporting HPV surveillance and vaccination impact monitoring;
- Introducing concepts of International Standards (IS) and secondary standards for HPV testing and their appropriate use;
- Providing guidance on specimen collection and handling for HPV testing;
- Providing an overview of HPV serologic and DNA testing and providing example protocols evaluated by the WHO HPV LabNet; and
- Providing guidance on laboratory quality assurance (QA).

The manual should be useful for all audiences involved in development and implementation of HPV vaccines, particularly those involved in generating or using HPV laboratory data. This document is a living document that will be amended in light of the future advances made in the area and future experience of laboratory surveillance and vaccination monitoring in a global sense.
2. HPV LabNet – Recent significant achievements

Some more recent, current and future activities/achievements of the HPV LabNet include:

- Funding successfully obtained by *Regional Reference Laboratory (RRL)/Japan* (via the Japanese Ministry of Health, Labour and Welfare) to conduct HPV surveillance activities and associated HPV LabNet work.
- Recent running of an international training workshop on HPV genotyping and HPV 16/18 serology by the *RRL/Thailand* (see point 3 below);
- The 3rd HPV LabNet proficiency study for HPV genotyping: DNA panels were sent out by *GRL/Sweden* in mid-Sept 2009; returned data are being evaluated.
- Confirmatory HPV genotyping of laboratory samples by the two *GRLs* is effectively in motion; all RRLs in the HPV LabNet have submitted samples as requested.
- Phase 2 HPV serology study (and 1st proficiency study for HPV serology) (VLP-ELISA) to soon commence, after extensive characterization/evaluation of several sources of public-donated VLPs.
- A pilot study on data reporting commenced recently (coordinated by *GRL/Sweden*) in HPV LabNet with the aim to develop a standardized common data reporting format (recommended by the 2nd WHO HPV LabNet meeting, Geneva, Switzerland, 17-19 Nov 2008) establishing which variables to register/report, the format for registration, and how to compile data by exchange of experiences within the LabNet.

3. HPV LabNet Training Workshops

The HPV LabNet *RRL/Thailand* recently conducted a successful Training workshop on HPV genotyping and HPV 16/18 serology, National Cancer Institute, Bangkok, Thailand, 3rd – 7th August, 2009.

- This was supported and facilitated by GRL/Sweden, RRL/India, RRL/Switzerland and WHO/Headquarters (HQ), WHO Regional Offices in Western Pacific and South-East Asia Regions (WPRO, SEARO), International Union Against Cancer (UICC) Fellowship and Dr. Morag Ferguson (Former principal scientist/NIBSC).
- The target audience of the workshop was scientists, technicians and public health personnel working or interested in developing HPV assays (particularly supporting HPV surveillance and vaccine impact monitoring).
- Attendance included 37 participants from both local (Thailand) and international (Indonesia, Republic of Korea and Bhutan) HPV laboratories.

Objectives:

- Provide practical and theoretical training on HPV genotyping and serology testing to personnel from hospitals/institutes/centers concerned with cervical cancer in Thailand and countries in South-East Asia Region (SEAR) and Western Pacific Region (WPR);
- Disseminate knowledge on HPV and HPV vaccines, international standards/reference reagents, quality control/assurance of HPV assays, HPV surveillance and vaccination impact monitoring and to train the participants on basic assays for HPV genotyping and serological measurement;
- Initiate the HPV laboratories networking in Thailand and potential countries in SEAR in order to standardize and harmonize HPV testing in the region to support HPV surveillance and vaccination impact monitoring;
- Prepare for future HPV vaccine introduction.

* WHO HPV LabNet members’ list is updated in this Newsletter, Point 9.
Workshop Content:
- The workshop comprised of theoretical presentations and laboratory practice/demonstration. 
  (Agenda and presentations are available on: www.kmnci.com).

Theoretical component:
- Basic information about HPV and cervical cancer, HPV vaccines, HPV surveillance and vaccination impact monitoring;
- WHO strategies in facilitating HPV vaccine introduction, international standardization, role/activities of HPV LabNet, quality control/assurance (QA/QC) of HPV laboratory testing;
- Overview of HPV assays: HPV genotyping and serology;
- Introduction of the HPV Laboratory Manual, HPV proficiency and confirmatory testing.

Practical component:
- HPV genotyping was performed with the PGMY-CHUV assay (refer 4th Newsletter) on 17 patient samples (4 negative, 13 positive) and an International HPV16 DNA Standard.
- PCR products were evaluated by gel electrophoresis, positive samples genotyped using reverse blot hybridization assay, and results / troubles discussed.
- An ELISA test for HPV antibodies and HPV neutralization assay were demonstrated. Principle of the assays, preparation of virus-like particle (VLP) and pseudovirion (PsV), calculation and interpretation of the test results were explained.

Results:
- Course evaluation questionnaire was distributed to all participants at the end of the training workshop, with feedback received from 25/37 participants;
- The course was found to be very interesting, useful, easy to understand, and paced appropriately;
- Trainers were all well-prepared and responsive to the participants learning needs;
- The only complaint being that not all participants could join in the practical component (due to the laboratory capacity limitation, 16 participants were selected to be trained in the practical laboratory sessions while the others followed the laboratory training via live video in the conference hall).

Conclusions:
- The workshop provided a great opportunity in communication, trouble-shooting and networking among regional reference laboratories and national/local laboratories.
- HPV surveillance and vaccine impact monitoring were discussed and initiated.
- Participants were interested in using International Standards/Reference Reagents and working standard control for HPV DNA and serology assays.

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Upcoming HPV LabNet training workshops

- The overall package of training program and presentations was shared with HPV LabNet members and comments invited for any improvement. It is envisaged that this experience may provide the basis for developing "standard" training curriculums within the HPV LabNet for use in future trainings.

- All RRLs are encouraged to disseminate HPV technical advice, knowledge and share experience via such training workshops to other labs within their geographical region.

- In addition to the WHO/HPV LabNet assistance/support, the UICC have expressed their interest in offering opportunities under their fellowships to support training and capacity building conducted by HPV LabNet. More information can be found on the link below: http://www.uicc.org/index.php?option=com_content&task=view&id=16&Itemid=116

- Future HPV LabNet workshops being planned:
  - RRL/Argentina is planning a similar theoretical and practical HPV course (in Spanish) in the Pan American Health Organization (PAHO) region in April 2010, with assistance from WHO/HQ and WHO/PAHO. Main objective is to harmonize and standardize laboratory procedures and create a Regional HPV laboratory network in order to support an effective surveillance system that provides evidence for the burden of HPV-related diseases and HPV type distribution in cervical cancer and pre-cancer cases in relation to HPV vaccine related-types. Participants will be from countries with public health laboratories active in HPV testing.
  - WHO/HQ is organizing "The 2010 WHO HPV LabNet Training Workshop on HPV Genotyping and HPV Serology Laboratory Performance"* in CHUV, Lausanne, Switzerland, 15-18 March, 2010. This will be hosted by RRL/Switzerland and facilitated by GRLs/Sweden and CDC, US and Dr. Morag Ferguson. Workshop content includes:
    - Knowledge on HPV and HPV vaccines; HPV surveillance and monitoring;
    - Implementation of international standards & preparation of secondary standards; QA/QC of HPV testing
    - Specimen collection; Overview of HPV testing options (DNA and serology)—strengths and limitations with focus on training laboratory performance of HPV genotyping and serology.
    - Sharing information/experience and identifying future needs from countries.

* Participants will be nominated by WHO Regional Offices. Due to limitation of laboratory capacity, 1 participant per Region is requested. It's anticipated that the participants will be able to assist the respective RRL provide technical support to other labs in the Region after attending the training workshop. Networking in future HPV work is also anticipated.

HPV Vaccine Biologic Outcomes Monitoring – a new subcommunity

If you are interested in participating in this and other discussions on the monitoring of HPV vaccines, you can join this subcommunity by using the following link:

Log into the Global Community Website (http://hpv-vaccines.net).

The initiating topic was: “What biologic outcomes should be/are being monitored for the HPV vaccine?”
4. Typical Activities of an HPV LabNet Regional Reference Laboratory: Tunisia

The HPV LabNet RRLs perform a number of tasks throughout the year related to HPV detection and surveillance, in and around their geographical location. Typical activities of the RRL in the Eastern Mediterranean Region (EMR) are:

**Enlarging the HPV infection prevalence survey**
- Since working on HPV prevalence surveys with hospitals for 6 years, the laboratory has introduced (in the past 6 months) HPV testing as a routine test available for all Tunisian women.
  - Sampling is performed at the Pasteur Institute with liquid based cytology.
  - Locally produce an efficient liquid, in an effort to make the test cheaper.

**Collaborative study with health institutions**
- In collaboration with national pathology laboratories, have initiated an HPV genotyping study among high-grade and invasive cervical cancer cases throughout Tunisia:
  - Formalin fixed and paraffin embedded samples \( n = 350 \) have been collected;
  - DNA has been extracted, with genotyping ongoing (Linear Array assay);
  - Results obtained will also serve to participation in the confirmatory testing by GRLs aiming to identify possible testing problems among the wide variety of samples encountered from clinical-epidemiologic studies worldwide.

**Information exchange and promoting HPV testing**
- On April 4\(^{th}\), 2009, a Seminar covering HPV infection, contamination, prevention, vaccines, ethical and social considerations was organized by the RRL/Tunisia (Pasteur Institute of Tunis).
  - All gynecologists of Tunisia, members of the national department of basic health (ministry of health) were invited (with 140 participants overall);
  - Outcomes were very positive;
  - Presentations were interactive and provided a better knowledge and communication amongst the attendees.

- The laboratory’s capacity of RRL/Tunisia for HPV genotyping and commitment with the WHO is well known across Tunisia, and it is hoped this will encourage collaboration with other laboratories.
- Willingness for HPV screening has improved; with numbers of cervical samples for HPV genotyping increasing within the laboratory.

**HPV research studies**
- HPV research has involved the study of interactions between HPV and NFkB (nuclear factor kappa beta) signalization pathways among cervical cancerous lesions. NFkB is a transcription factor playing an important role within the host immune system.

The Scottish HPV Reference Laboratory (SHPVRL) was established as part of a national cervical cancer prevention strategy after introduction of a schoolgirl HPV immunization programme in mid-2008.

- A National HPV Steering Group was set up in the previous year to:
  - Oversee introduction of the programme;
  - Establish adequate epidemiology and surveillance structures;
  - Develop good communication and education packages;
  - Form local implementation groups linked to the Scottish Cervical Screening Programme (SCSP)

- The SCSP starts with a centrally organized personal invitation sent to all women before their 21st birthday, which has yielded effective delivery of many aspects of the programme.

- The HPV Vaccine Programme is for all girls aged 12-13 years old, with catch-up to age 18 spread over 2 years (Scotland) or 3 years (England).

- The communication strategy has proven highly effective winning TV advert awards, and the schoolgirl programme achieving over the target 85% coverage in its first year (Table).

- SHPVRL is evaluating baseline HPV prevalence data before immunized girls enter the SCSP.

- In the past year, SHPVRL has looked at HPV type prevalence among:
  - Teenagers (using urine as a non-interventional specimen);
  - Women attending their first cervical smear (PreservCyt LBC samples);
  - Women not attending screening (self-collected swabs/urines and postal kits);
  - Cervical cancers pre-2005.

- A similar exercise will be repeated to assess the impact of vaccination, particularly in women entering the screening programme, and finding ways to reach those who default.

- Additional work by the SHPVRL has included:
  - Prepare for changes in SCSP, such as HPV testing after lesion ablation to reduce the intensity of follow-up;
  - Carry out HPV triage if introduced nationally and to cross-train current cytology technicians in molecular HPV technologies;
  - Provide HPV testing to aid clinical management, not only of cervical disease but of head and neck cancers, advise on other areas where HPV testing might be useful.

- Quality assurance of all HPV testing might be undertaken within the National Health Service.

- Scotland’s national Reference Laboratories are commissioned by Health Protection Scotland to address areas of public health need and funded by Health Board budgets by the Scottish Government Health Department.

- First year of operation has been fruitful and will inform future vaccine use and the future organization of SCSP.

- To compliment the Reference Laboratory facility, the Scottish Government’s Chief Scientist Office has funded two major programmes of research covering biotechnology and health services research over the next five years.
6. Meeting Updates

Cervical Cancer – Not Yet Beaten!

July 24th, 2009, Bio21 Institute, Parkville VIC, Australia

A one-day HPV vaccine forum, (initiative of AOGIN) was conducted by RRL/Australia covering a variety of HPV-associated topics:

- HPV vaccines;
- HPV DNA testing;
- Changes and progress in cytology screening;
- Other HPV related cancers.

Keynote speaker – Prof. Margaret Stanley (UK), and other International speakers [Prof. Peter Stern (UK), Dr. S. Quek (Singapore)]

25th International Papillomavirus (IPV) Conference & Clinical Workshop

May 8th -14th, 2009 Malmö, Sweden

This IPV meeting comprised of 2225 registered participants, with more than 2000 being foreign delegates from 80 different countries.

The Conference/Workshop encompassed a wide range of aspects of Papillomavirus research, from clinical vaccinology to molecular biology, comprising of 887 posters and 237 oral presentations.

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

Presentation highlights include:

- Increasing importance of tumor virology in explaining causes of cancer and providing new methods for its prevention;
- Rates of genital warts are declining in Australia, just a year after introduction of an HPV vaccination program;
- The higher the prevalence of HPV, the more common is cervical cancer;
- Cervical screening with sampling for HPV tests at home improves coverage by 10%;
- New mechanisms for detecting HPV vaccines: the small protein L2 of the virus capsid may offer less expensive vaccines with broader protection against more HPV types;
- Men have particularly high rates of HPV infection: as many as 5% of men get HPV / month;
- Do allergies protect against HPV-associated cancer?
- Who should be vaccinated and how? How can we estimate which HPV strategy will be most effective for eradication of HPV and HPV-associated cancers?
WHO HPV Surveillance and Monitoring Meeting

November 16th - 17th, 2009, WHO/HQ, Geneva, Switzerland

Preface: New approaches to monitoring vaccine coverage, vaccine safety, and biological impact are required; particularly prior to administering HPV vaccines into populations not previously been routinely served by Expanded Programme on Immunization (EPI). HPV outcome monitoring involves engaging partners in the fields of immunization, sexually transmitted infections, reproductive health, adolescent health, and cancer.

Objectives: To further delineate objectives and strategies, partners, and resources for HPV vaccine impact monitoring. These include:

- HPV vaccine impact, including short term (HPV prevalence studies), medium term (precancerous lesions surveillance), and long term (cancer registries);
- HPV vaccine coverage;
- HPV vaccine safety;
- And to discuss / identify:
  - Laboratory needs for HPV vaccine impact monitoring;
  - Partners and resources for HPV surveillance and monitoring.

Expected outcomes:

- Agree on the architecture of a guidance on monitoring HPV biologic endpoints, (incl. to identify optimal sentinel surveillance sites, tests to be used, and strategies to implement surveillance for biologic endpoints:
  - Prevalence of HPV infection
  - Positive cervical cancer screening tests and treatment referrals for cervical abnormalities,
  - CIN 2-3, including associated HPV types
  - HPV type distribution in cervical abnormalities
  - Invasive cancer, including associated HPV types;
- Decide if genital wart monitoring is feasible, and if so, outline guidance on how to do it;
- Decide if non-cervical cancer monitoring is feasible, and if so, outline guidance on how to do it.
- Discuss possible approaches to HPV vaccine coverage monitoring,
- Review HPV vaccine safety monitoring,
- Identify and prioritize special studies that may be needed to provide more complete guidance.

26th International Papillomavirus Conference & Clinical Workshop

July 3rd - 8th, 2010, Montréal, Canada
More information can be obtained on the link: http://hpv2010.org/main

AOGIN – 2010 India

March 26th-28th, 2010, New Delhi, India
More information can be obtained on the link: http://www.aoginindia.org

EUROGIN – Cervical Cancer Prevention

February 17th-20th, 2010, Monte Carlo, Monaco
More information can be obtained on the link: http://www.eurogin.com/2010
7. Useful Web Links

- http://www.who.int/immunization/en
- http://www.who.int/biologicals/en
- http://www.who.int/hpvcentre/en
- http://www.uicc.org
- http://www.ipvsoc.org/index.html

8. Contributions for Next Edition of HPV LabNet Newsletter

The WHO HPV LabNet newsletter is widely distributed for a public audience, including:

- WHO regional offices for broad dissemination;
- WHO/Immunization Vaccines and Biologicals department and internal partners in other departments (e.g. Cancer Control, Reproductive Health and Research, etc) for broad dissemination;
- External partners (e.g. ICO, UICC, IARC, participants at previous WHO HPV meetings, etc);
- Posting on the WHO website, ICO website, and websites of HPV LabNet members

Any suggestions on other avenues for wider dissemination of this newsletter are most welcome.

The 6th WHO HPV LabNet Newsletter [to come in the 1st half of 2010]

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.

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