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Meeting Report

WHO HPV LabNet Training Workshop on HPV Genotyping and HPV Serology Laboratory Performance

Lausanne, Switzerland

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EXECUTIVE SUMMARY

A theoretical and practical training workshop was organized in the Regional WHO HPV Reference Laboratory (RRL, Europe), in Lausanne, Switzerland, on 15-18 March 2010, to provide knowledge and information on all aspects involved in good HPV laboratory testing performance including practical performance of proficient HPV genotyping and serology assays. The participants were from countries where HPV laboratory testing is being actively set up. Implementing good performance of HPV laboratory testing and international standardization will ensure that proficient and standardized assays are used in HPV epidemiological studies and reliable data are generated to promote international harmonization of HPV laboratory testing and comparability of data across different laboratories. Information on HPV laboratory activities to support HPV surveillance and monitoring in respective countries were presented by the participants. A comprehensive HPV Laboratory Manual (developed by the WHO HPV Laboratory Network) and critical materials were provided to the participants to take back to set up new assays in their laboratories. Participants regarded this training very informative, helpful and in good timing when they are setting up HPV laboratory testing capacity in the countries to support HPV epidemiological studies. Networking between national HPV laboratories with the WHO HPV LabNet was promoted.

1. Background

Human Papillomavirus (HPV) laboratory surveillance and vaccination impact monitoring is a critical element in the process of HPV vaccine introduction. The World Health Organization (WHO) established the WHO HPV Laboratory Network (HPV LabNet) in 2006 to harmonize and standardize laboratory testing procedures to support consistent laboratory evaluation of regional disease burden and monitor the performance of HPV vaccines. Significant progress has been made in the area of standardization of HPV laboratory testing to promote international harmonization including development of international standards, evaluation and standardization of HPV assays (DNA genotyping and antibody measurement), development of Quality Assurance (QA)/ Quality Control (QC) program, capacity building and training. Requests for technical support on HPV laboratory testing have been received from countries.

In response to requests, WHO organized The 2010 WHO HPV LabNet Training Workshop on HPV Genotyping and HPV Serology Laboratory Performance, at the

Regional Reference Laboratory (RRL) of HPV LabNet, Institute of Microbiology, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland, on 15-18 March 2010.

2. Participants

The workshop was attended by 15 participants from 12 countries. Technical facilitation was offered by Professor Joakim Dillner [Global Reference Laboratory (GRL)/HPV LabNet], Dr Elizabeth R. Unger (GRL/HPV LabNet), Dr Morag Ferguson, Dr Iwao Kukimoto (RRL/HPV LabNet), Professor Denise Nardelli-Haeffliger and Dr Roland Sahli and staff in CHUV, RRL/HPV LabNet.

The trainees in this workshop were nominated by WHO Regional Offices and from countries where HPV laboratory testing is actively setting up to support HPV surveillance, namely Argentina, India, Iran, Italy, Morocco, Republic of Korea, Uganda (see list of participants in Annex 1).

3. Workshop/practical course

The workshop program included consecutive sessions of theoretical presentations and discussions with intermittent practical laboratory work as elaborated in Annex 2, which covered all aspects involved in good HPV laboratory testing performance of HPV DNA genotyping and serology assays.

HPV DNA detection and serology assays play essential role in HPV epidemiological studies of HPV surveillance and vaccination impact monitoring. During the practical training sessions of this workshop, two basic HPV assays, one DNA genotyping and one serology, that were verified by the WHO HPV LabNet and detailed in the HPV Laboratory Manual (developed by the HPV LabNet and to be published) were performed, namely:

- An in-house HPV DNA genotyping assay: PGMY- reverse blotting hybridization (RBH), namely "CHUV" assay, with use of patient cervical cytology samples
- A virus like particle (VLP)-based enzyme-linked immunosorbent assay (ELISA) for HPV16 antibodies with use of patient sera and the International Standard (IS) of anti-HPV16 sera

In addition, an HPV in vitro neutralization assay, as detailed in the HPV Laboratory Manual, was presented in the format of a laboratory demo.

TQ Zhou (WHO, Geneva, Switzerland) outlined the objectives and expected outcomes of this workshop at the start. She first gave a brief background for organizing this workshop. HPV vaccine introduction has been facilitated by WHO. HPV laboratory surveillance and vaccination impact monitoring is a critical element in the process of HPV vaccine introduction. WHO established the HPV LabNet to harmonize and standardize laboratory testing procedures to support consistent laboratory evaluation of regional disease burden and monitor the performance of HPV vaccines (<http://www.who.int/biologicals/vaccines/hpv/en/index.html>). Significant progress has been made in the area of standardization of HPV laboratory testing to promote international harmonization including development of international standards, evaluation and standardization of HPV assays, development of Quality Assurance (QA)/Quality Control (QC) program, capacity building and training. One of the roles of the HPV LabNet is to provide technical support to national laboratories to ensure the availability of competent laboratory services worldwide for HPV DNA and antibody detection. Such requests for technical support on HPV laboratory testing have been received from some countries. In response to the need, WHO/HPV LabNet organized this workshop to provide theoretical and practical knowledge on all aspects relating to good performance of HPV laboratory testing to support HPV surveillance. The target audiences were the laboratories from countries worldwide that are active in HPV laboratory testing to support HPV surveillance, and were nominated by each WHO Regional Office.

The main **objectives** of this workshop were to inform participants about WHO synergy work in facilitating HPV vaccine introduction and share experience of WHO HPV LabNet as well as to train the participants on the following aspects: basic/general knowledge of HPV biology and HPV vaccines, concept and rationale of international standardization, theoretical and practical knowledge of HPV genotyping and serology assays (through presenting an overview and rationale of variety of assays, practicing example assays verified by the WHO HPV LabNet), principles and knowledge of HPV laboratory QA/QC. This workshop also provided an opportunity to share experience from national laboratories in providing laboratory support to HPV surveillance and vaccination monitoring in the countries in order to identify needs for further support and to promote

networking in conducting HPV surveillance and vaccination monitoring. Through attending this workshop, participants would acquire knowledge on the good performance of HPV laboratory testing and international standardization to ensure that proficient and standardized assays are used in HPV epidemiological studies and reliable data are generated that would promote international harmonization of HPV laboratory testing and comparability of data across laboratories; communication would be bridged towards networking in HPV surveillance and vaccination monitoring among countries and with the WHO HPV LabNet; capacity of participating laboratories in performing proficient HPV DNA genotyping and serological assays would be built/ strengthened to support HPV surveillance and vaccination monitoring in the countries. It is anticipated that the participants will be able to assist the RRLs of the WHO HPV LabNet to provide technical support to other national laboratories in the respective WHO Region, when such need is identified, and to disseminate knowledge.

E Unger (HPV LabNet GRL, Centers for Disease Control and Prevention, USA) gave a thorough presentation on the knowledge of HPV biology and disease burden, including basic knowledge of HPV types, viral cycle and natural history. She also presented the development of HPV prophylactic vaccines, as well as the latest progress and future challenges.

TQ Zhou gave in a second presentation a snapshot on the WHO synergy activities in facilitating HPV vaccine introduction, with focus on progress made in the international standardization of HPV by the WHO HPV LabNet.

WHO has long been facilitating the development and introduction of HPV vaccines in the world through synergy work which is a very comprehensive and programmatic project and contributed by multi-departments/teams at WHO through close collaboration with external partners. WHO facilitates the HPV vaccine introduction through 1) **Policy development** to provide evidence-based guidance to support and facilitate decision-making in countries (1-7); 2) **Programmatic support** to provide technical support and education, promote sharing information, disseminate scientific knowledge, facilitate international harmonization in all related aspects through development of international standards and consensus, ensure that safe and efficacious HPV vaccines are used in countries through setting up international specifications on vaccine quality, safety and efficacy and WHO pre-qualification programme, provide support to post-vaccination

monitoring and support capacity building in countries etc; 3) **Support to research** to monitor and facilitate technical advances and generate scientific evidence in HPV vaccine implementation; 4) **Communication** by convening international consultations, workshops and forums at global level, in WHO Regions and in countries; establish dialogues with countries, regulators, public health agencies, vaccine developers, manufacturers, suppliers, partners etc. to promote the education and communication in HPV vaccine implementation.

Based on scientific evidence and expert recommendations, in April 2009, WHO published the recommendations on HPV vaccination, namely WHO Position Paper (8, 9). Setting norms and standards and promoting their implementation are WHO core functions. These norms and standards are international technical specifications for the quality, safety and efficacy of a wide range of biological medicines. WHO is committed to support countries to ensure that 100% of vaccines used in all national immunization programmes are of assured quality. WHO norms and standards include global written standards and measurement standards. The use of these standards will facilitate international harmonization of vaccine licensure and laboratory testing, facilitate comparisons of results between different laboratories and improve laboratory performance (10).

WHO developed Guidelines to Assure the Quality, Safety and Efficacy of Recombinant Human Papillomavirus Virus-Like Particle Vaccines in 2006 (4) to provide guidance for national regulatory authorities and manufacturers on production, quality control, non-clinical and clinical evaluation of HPV vaccines and support harmonization of international regulation of HPV vaccines. In these WHO Guidelines HPV DNA genotyping and serology assays are required in assessing HPV vaccines in clinical trials for different purposes. Moreover, HPV assays play crucial roles in conducting HPV surveillance during pre-vaccine introduction and post-vaccine introduction era. Unique features of HPV family complicate the HPV laboratory testing both in DNA and antibody detection. A variety of assay formats have been in use for HPV genotyping and antibody detection in worldwide laboratories. Studies have shown that lack of international standards for HPV serology, DNA detection, laboratory reagents and quality assurance procedures results in significant variations in test performance; and this has hindered both epidemiological studies of HPV infection and comparison of results from different HPV vaccine trials (11, 12). In order to meet the need for international standardization, WHO

established the HPV LabNet in 2006, as part of the project of "Generating an enabling environment for HPV vaccine development and global introduction" with funding from Bill & Melinda Gates Foundation for a period of 2005- 2010. The HPV LabNet aims to harmonize and standardize laboratory testing procedures to support consistent laboratory evaluation of HPV disease burden through facilitating the implementation of validated, standardized laboratory procedures, developing quality assurance and proficiency testing, training personnel and providing a network for surveillance. Up to date, the WHO HPV LabNet comprises of 2 GRLs and 8 RRLs among the six WHO Regions. Over the past years, the HPV LabNet has been making efforts in several areas, in close collaboration with the WHO International Laboratory for Biological Standards, National Institute for Biological Standards and Control (NIBSC), UK, including **1) Development and implementation of International Standards (IS)**. The 1st IS for anti-HPV type 16 sera and 1st ISs for HPV type 16 and 18 DNA were established (13) and available to worldwide laboratories. There are several other ongoing projects of development of HPV standards. **2) Harmonization and standardization of HPV assays**. The HPV LabNet has identified, evaluated promising assays; developed consensus on standardized protocols and QA/QC programs (e.g. Proficiency Study, Confirmatory testing); developed an HPV Laboratory Manual to provide basic knowledge and guidance and technical help to needed labs. Priority was given to the assays that are reliable and proficient, easy to perform, easy to transfer and implement in all resource settings (e.g. low resource), and cheaper cost/ affordable by low resource settings. This will assist and support worldwide labs, in particular in developing countries, to set up reliable HPV assays, standardize laboratory testing in HPV epidemiologic studies, and allow inter-laboratory comparison of results. **3) Capacity building**. Laboratory testing capacity has been strengthened through collaborative studies, training, technology transfer, follow-up to proficiency study and exchanging information/knowledge to ensure basic, reliable HPV genotyping and antibody detection capacity available in each RRL and advanced capacity in GRLs, to be competent for providing technical support to national labs to meet the upcoming need. **4) Technical consultations**. HPV LabNet laboratories have been deeply involved in technical consultations at all levels and have provided scientific and technical advice to decision-making process; conducted HPV epidemiological studies; and provided trainings, technical support to other laboratories in countries. **5) Information exchange**. Useful tools were established to provide platforms for exchanging information, sharing knowledge, seeking technical advice, harmonizing and

improving HPV laboratory practice, exploring collaboration and synergy of work, which include meetings, HPV LabNet Workstation (SharePoint), WHO HPV LabNet public website and Newsletters.

Through 4 years of effort, the HPV LabNet has been able to provide service in HPV laboratory testing via assisting in evaluation and validation of assay methodologies used in vaccine evaluation, surveillance and monitoring to allow inter-laboratory comparison; providing standards and standardized assay protocols for use in HPV surveillance and monitoring to promote data comparability cross different laboratories and epidemiologic studies; providing QA/QC programs, e.g. Proficiency Panel, Confirmatory Testing to ensure reliable and proficient assay performance in HPV epidemiology studies; providing specialized laboratory expertise, technology transfer, advice, support and training to needed laboratories; providing service for testing surveillance samples; sharing knowledge and information via WHO HPV LabNet website, meeting reports, publications, SharePoint, Newsletters, HPV Laboratory Manual, etc; and providing fundamental infrastructure to support future "expanded" network for HPV surveillance.

An HPV laboratory serving a country is required to set up appropriate assays that are proficient, reliable, fit for purpose/use (considering the available resource and real need), to validate and standardize assays to ensure results be traceable to ISs, to build the laboratory capacity, maintain staff training to ensure good practice/performance, and to implement laboratory QA/QC programs to ensure the quality and consistency of data generated. The current established knowledge, e.g. WHO resources, HPV Laboratory Manual, shall be referred and used. In conducting HPV surveillance in the countries, it is encouraged to establish collaborations with HPV LabNet RRL and WHO/Regional Office via networking. Useful WHO resources related to HPV vaccination and cervical cancer were provided to the participants.

At the end of Dr Zhou's presentation, acknowledgements were given to the members of WHO HPV LabNet and NIBSC, UK for their hard work in making the achievements, and to the Bill & Melinda Gates Foundation for providing financial support to the project.

E Unger presented the different HPV testing options and indications, including the different assays allowing detection of HPV DNA and/or HPV typing as well as serological assays. She explained the difficulty linked to the establishment of the latter due to the lack of commercial assays and/or critical reagents (e.g. VLPs). An overview of

the content of the WHO HPV Laboratory Manual, as well as its prospective use was presented. The manual includes chapters of 1) Introduction; 2) Role of the Laboratory in HPV Surveillance and Vaccine Impact Monitoring; 3) Laboratory Quality Assurance (includes safety); 4) Collection and Handling of Specimens for HPV Testing; 5) DNA Extraction and HPV DNA Testing; 6) Assay Validation; 7) HPV Serology – ELISA Assay; 8) HPV Neutralization Assay; 9) International Standards and Secondary Standards; and 10) Data Management. The manual provides brief summary of HPV biology and natural history and burden of disease, discusses the role of laboratory in HPV surveillance and vaccination monitoring, introduces ISs and secondary standards for HPV testing and their appropriate use, provides guidance to specimen collection and handling, gives an overview of HPV testing and example protocols evaluated by the WHO HPV LabNet, provides guidance on laboratory quality assurance. The current draft version of the manual, currently under WHO review for final approval, was distributed to the participants for their use during the workshop. It was emphasized that the manual is a "living" document that will be frequently updated to reflect current best practice and scientific advances.

J Dillner (HPV LabNet GRL, Lund University, Sweden) presented the key issues of HPV vaccination monitoring and highlighted the importance of laboratory testing to survey potential HPV type replacement and determine overall vaccine efficacy in a population, especially when vaccine and disease registry are lacking. Vaccination monitoring strategies were further discussed including examples from Nordic countries. In summary, vaccination monitoring is necessary due to asymptomatic HPV infection and long lag between infection and disease. Basic laboratory services will have to continue phase IV/V trials and/or test vaccines designed for use in developing countries, establish basic epidemiology of infection and disease burden and make early evaluation of effectiveness in HPV testing surveys. In some countries, advanced evaluation programs with registries, reporting systems, registry linkages etc will have to be performed.

J Dillner then introduced HPV DNA proficiency testing and how this could be achieved by using proficiency panels and ISs. The 2008 WHO HPV DNA proficiency panel was tested by 54 laboratories worldwide that used different assays. The most widely used method that yielded good performance was the commercial Linear Assay. PGMY-RBH ("CHUV" assay) was shown to be an acceptable non-commercial alternative due to its low cost. Several methods, e.g. polymerase chain reaction (PCR)-Luminex, type-specific

PCR, PCR-Chip) yielded good results, but were used in only one or a few laboratories. Globally, internationally standardized evaluation of performance (assay method and laboratory performance) was shown to be feasible. In order to share the information with the public, a detailed description of the study is being prepared for publication.

R Sahli (HPV LabNet RRL for Europe, CHUV, Switzerland) described the PGMY-RBH ("CHUV" assay) and its validation, as well as the experience gained from technology transfer within the WHO HPV LabNet and from participation in WHO proficiency testing that helped improve the original standard operating procedures (SOP) and resulted in the final description of this assay in the HPV Laboratory Manual.

The assay was developed with the PGMY primer sequences provided prior to publication by P. Gravitt and colleagues in 1999. A probe array on a nylon membrane was designed for HPV type-specific detection that allowed multiple uses (at least 10 times and up to 28 times) to reduce the cost of typing as much as possible. Probe adequacy was proven over 10 years of typing (9,500 samples) with an estimated level of hybridization-negative/PCR-positive samples associated with mismatches within probes as low as 1% (sensitivity = 99%) as determined by DNA sequencing and absence of cross reactions (specificity = 100%). The distribution of types affected by mismatches was unequal and targeted mainly known subtypes of HPV types 45, 51, 68 and 82. Based on this sequence information a new version of the probe panel is under prospective evaluation with good results up to now.

The initial concentration of magnesium chloride ($MgCl_2$) in the PCR (1.5 mM) was established with sensitivity threshold of at least 50 copies per reaction using DNA from known amounts of HPV16 and 18 positive cell lines mixed in an excess of HPV negative cells. This $MgCl_2$ concentration was also selected based on the good discrimination of positive/negative cases by gel electrophoresis using cytology as gold standard early in the assay development. Participation to proficiency testing revealed, however, a suboptimal sensitivity (> 500 copies of HPV 31, 33, 35 or 56 per reaction) for epidemiological purpose. Re-evaluation of the $MgCl_2$ concentration established 3 mM as optimal for most types despite a bad signal to noise ratio at gel electrophoresis with a high frequency of doubtful cases that were indeed negative after hybridization or sequencing. The simultaneous use of the two different concentrations of $MgCl_2$ led to a further increase in sensitivity, especially with mixed infections (prospective analysis of 827 samples).

Further data on a few hundred samples were presented and confirmed that the PGMY-RBH ("CHUV" assay) is reproducible under intra-laboratory conditions and comparable to the commercially available Linear Array assay from Roche.

Key points about the SOP were also discussed underscoring: 1) the importance of strictly following each step, in terms of reagents as well as in terms of membrane handling; 2) that the probe manufacturing process (and the manufacturer) must be carefully selected to eliminate the risk of probe cross contamination; and 3) that additional and strict quality control procedures must be followed in comparison to a commercial assay to ensure reliability of this in-house assay. Finally, participation to the proficiency panels was shown to have a major impact on improving the technique for epidemiological purpose.

R Sahli and C Estrade (HPV LabNet RRL for Europe, CHUV, Switzerland) demonstrated the PGMY-RBH ("CHUV" assay) on 36 samples previously amplified and typed for diagnostic purpose in Dr Sahli's laboratory. The 8 participants were distributed in two groups so that each could actively apply samples in the electrophoresis system and very importantly apply samples on two array membranes, each in one miniblotted. This step was particularly informative as it needed to be repeated by some participants with buffer only to get the necessary skill to avoid sample cross contaminations. This step was representative of probe loading at membrane array setup that was otherwise not performed for sake of time. Gel electrophoretic results were discussed the first day, illustrating the role of positive, negative and internal human DNA controls, the "matrix" PCR setup and the somewhat higher background but overall higher PCR signal obtained with 3.0 mM MgCl₂. RBH was performed the next day according to the SOP (that were distributed to each participant). During the incubation time, C Estrade and R Sahli shared their experience with the participants, emphasizing the role of each step of the SOP with illustrative examples where necessary. The absence of membrane background confirmed their reusability (one was used 4 times and the other 2 times), and all samples that were typed during this workshop gave expected results, consistent with the good reproducibility of the technique despite application of the samples by different individuals.

I Kukimoto (HPV LabNet RRL for the Western Pacific, National Institute of Infectious Diseases, Japan) presented his experience with the PGMY-RBH genotyping

assay ("CHUV" assay) as a routine HPV typing method for clinical samples. The RRL, Japan introduced the assay as a result of the HPV LabNet technology transfer in 2008..

To set-up the "CHUV" assay, four processes were taken in a stepwise fashion. Firstly, L1 PCR using degenerate PGMY11/09 primers was established with standard HPV DNAs that were provided by the European RRL. Secondly, these PGMY PCR products were subjected to RBH with a quality-assured membrane provided by the European RRL. Thirdly, a new membrane was prepared by Dr Kukimoto's laboratory and used in RBH with the same PGMY PCR products, the result of which exhibited almost identical results between the two membranes. Lastly, clinical DNA samples isolated from Japanese CIN patients were examined by the "CHUV" assay using the in-house membrane. Dr. Kukimoto's laboratory used the "CHUV" assay with 3 mM MgCl₂ to test samples in the 3rd HPV DNA proficiency panel in 2009. The proficient results indicated that the introduced "CHUV" assay had the sensitivity and specificity required for HPV surveillance as a reference laboratory.

HPV genotyping of clinical samples by the "CHUV" assay has started in the RRL in Japan. Results with cervical DNA samples from 393 patients that had visited the Nippon Telegraph and Telephone Medical Center, Tokyo from November 2009 to February 2010 have shown 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. Sequencing of PCR-positive and hybridization-negative samples revealed types that were not included in the probe panel on the membrane, HPV types 61, 62, 67, 71, 74, 81, 86, 87, 89, 90, which verify the specificity of hybridization detection.

In conclusion, the "CHUV" genotyping assay is a very sensitive, robust and reliable method to determine HPV types in clinical samples at affordable cost. The RRL in Japan is going to collect further data on HPV prevalence in Japan using the "CHUV" assay.

J Dillner introduced HPV serology assays and their potential use. The basic principle of vaccination and how antibody assays can be used were reviewed. The different serological assays were presented and findings obtained in sera from naturally infected individuals and from vaccinees were reviewed. J. Dillner then thoroughly presented the

WHO HPV LabNet collaborative studies to assess comparability of HPV antibody measurements. The first phase study was performed in 10 laboratories without attempts to standardize the methods and yielded variable results, although transformation of antibody levels towards the same reference serum using the parallel line (PLL) method led to more uniform data. The use of the IS of anti-HPV16 sera together with the PLL method was advocated.

J Dillner briefly presented the principle of HPV neutralization assay and the production of VLPs and pseudovirion (PsV). Most VLPs are now produced similarly to PsV in mammalian system using codon-adapted plasmids and can be used for the more easily transferable ELISA. VLP-ELISA can be recommended for use by any laboratory interested in HPV serology, provided that WHO guidance on VLP quality control, SOP for ELISA are followed and data are reported in internationally comparable Units (all this information were described in the HPV Laboratory Manual).

J Dillner finally presented the essential steps towards the quality control of VLPs, which includes electron microscopy, gel electrophoresis, analysis of epitope exposure and ELISA testing with serum proficiency panels.

D Nardelli, M Bobst and S Alvarez (HPV LabNet RRL for Europe, CHUV, Switzerland) presented the PsV-based neutralization assay and explained different practical hints following the protocol detailed in the HPV Laboratory Manual. This assay is performed with PsVs that consist of type-specific HPV capsids that have encapsidated a reporter DNA that encode for secreted alkaline phosphatase (SEAP). Neutralizing antibodies are titrated according to their ability to prevent infection by PsVs of special target cells that stably express SV40 large tumour antigen (293TT cells). These cells allow replication of the infecting encapsidated reporter DNA and thus amplification of SEAP signal upon successful infection. In addition to the fact that this assay necessitates the production of PsVs, it also involves multiple critical steps during the 4-day assay period which make it less simple than a VLP-ELISA assay.

In the workshop, direct VLP-ELISA was practiced by the participants following the SOP detailed in the HPV Laboratory Manual using HPV 16 VLP-coated plates as well as serum samples provided by GRL Sweden. Eight participants tested the same serum samples including the IS of anti-HPV16 serum. The results obtained by all groups were discussed and compared. The PLL values calculated for each serum sample were very

similar among the HPV16 positive samples (inter-participants coefficient of variations between 20 and 28% depending on the samples), while one sample was found negative (PLL < 2) by all participants. This further demonstrated that this SOP/assay is robust and highly reproducible, at least when using the same VLP lot.

E Unger presented the different definitions and criteria QA and QC in laboratory testing. The guidance described in the HPV Laboratory Manual (Chapter 3) is a good source for building proper QA/QC system. How to handle QA/QC in the laboratory was further detailed including how to prepare a SOP. Laboratory safety, for which WHO laboratory safety manual exists, was also discussed. The importance of external or internal audit of the whole QA/QC system was emphasized. It was recommended that each HPV LabNet laboratory prepares QA/QC overview for the laboratory, share any tips they found helpful and share problems encountered.

M Ferguson (formerly Principal Scientist at NIBSC, UK) summarized why biological standards are required and outlined the preparation and establishment of ISs and how they are used. She went on to describe how antibody and Nucleic Acid Amplification (NAT) standards against a range of viruses have been used and then described in detail the studies on the development of the IS for anti-HPV 16 serum and ISs for HPV 16 and 18 DNA.

J Dillner explained that proficiency and confirmatory testing for HPV DNA is one of the roles of GRL, providing quality control on the entire testing chain. On request by WHO, certain samples were submitted by RRLs which were then tested by using different methods in the two GRLs. An example of testing results was presented and discussed. Some issues were identified such as: 1) a high concordance was found in particular between methods based on same principles; 2) for samples with multiple infections, it was difficult for all laboratories to reach consensus. This further demonstrated the need for accurate and broad genotyping methods. All results in the confirmatory testing are being analysed by the GRLs and a summary report will be concluded.

M Ferguson described in a second presentation how secondary or working standards should be prepared and calibrated against the IS. She described how standards facilitated consistent implementation of assays by laboratories, inter-laboratory comparisons, assay validation, on-going data monitoring of assay performance and reproducibility between

assay runs. The inclusion of a working standard in every assay also results in a reduction in laboratory errors.

M Ferguson also gave a presentation on assay validation in which she described what validation is, when tests have to be validated, the validation process and parameters to be considered. She also went into further detail on the validation of antibody assays and NAT assays and in the latter described the experiments and samples which should be undertaken to validate an HPV NAT assay.

E Unger presented several issues linked to specimen collection and processing for HPV detection. Dr. Unger went through the different types of samples and their use, as well as extraction methods giving some troubleshooting suggestions. She emphasized the need to carefully monitor and standardize all steps of sample collection and processing.

J Dillner discussed how data reporting and management should be performed with a focus on the particular needs within the HPV LabNet. The necessity for a common standard data reporting format was highlighted and the HPV LabNet is piloting this work.

In the last session of the workshop, all participants were given an opportunity to present their country's situation with respect to HPV vaccine implementation, HPV surveillance and vaccination impact monitoring, laboratory activities in supporting this work, and future work planned after this training course.

F Carozzi (ISPO- Istituto per lo Studio e la Prevenzione Oncologica, Italy) first presented the HPV vaccination status in Italy. In 2008 the Italian Ministry of Health (MoH) launched the HPV mass vaccination for 11 years old girls using active calling and providing the vaccine free of charge. The choice of vaccine is made regionally, so in Italy regions differ in which of the two vaccines are provided. According to the most recent data, HPV vaccine coverage is about 61% in the 1997 cohort (those girls who were born in 1997 and thus they were 12 years old in 2008). In Basilicata region, where the vaccination program covers cohorts of different age, the coverage decreases with age (~ 67% at 15 years, ~ 64% at 18 years and ~37% at 25 years.)

The MoH financed a set of studies aimed at assessing the pre-vaccine HPV epidemiology in women aged 25-60 participating in a screening program for cervical cancer (over 50.000 women), in young women aged 18-24 (4,000) and in 1,000 CIN2+ tissue samples obtained in Italy in the past 10 years. Dr Carozzi's laboratory was involved in all of these

studies by coordinating the procedures and HPV DNA genotyping. All the studies used the same methodology and involved ten different regions, so the data obtained can be considered representative of the entire national territory. Typing was performed using GP5+/GP6+ PCR primers that amplify a broad spectrum of HPV genotypes by targeting a 150-bp fragment within the L1 open reading frame of the HPV genome. The preliminary data (unpublished) on HPV type distribution in general population (18-60 years) showed a typical prevalence curve of HPV infection, with the peak in the age group of 21-24. There was no statistically significant difference between North, Central and South Italy. HPV 16, followed by HPV 31, 51, 56, 58, 52 are the most prevalent HPV types in normal population (without lesions). In a study on CIN2, CIN3 and cancer tissue samples, cases were sampled through the electronic databases of the pathology units and paraffin embedded tissue samples were collected from historical archives according to a standardized protocol. To overcome any false negatives due to inhibitors commonly present in formalin-fixed paraffin-embedded tissue, all HPV negative samples at this first step, were retested in diluted forms. To overcome misclassification of the HPV genotype resulting from potentially degraded DNA in aging archival paraffin-embedded tissues, for those samples that still remain negative for HPV, a second PCR-based strategy amplifying a shorter HPV DNA fragment was used. There was a statistically significant decreasing trend in the proportion of HPV 16/18 positive invasive cancers with age, decreasing from 92% in women aged <35 to 73% in women aged >55.

A multicenter project on "Effective surveillance and impact of HPV vaccination on screening for cervical cancer", a randomized study sponsored by public health and in collaboration with the WHO HPV RRL, Europe, is ongoing. The study will evaluate 1) the levels of HPV 16/18 antibodies and HPV type distribution at enrolment and at the next rounds of screening and 2) HPV prevalence in cervical samples compared with urine to evaluate the possibility to monitor HPV status in younger girls. Dr Carozzi also coordinates a working group (Italian Group of Cervical Cancer Screening, GISCI) to try to involve all the laboratories working in HPV testing in Italy in a quality assurance program.

Dr Carozzi, also shared the experience she gained in participating at different meetings of the WHO HPV LabNet since June 2007, and in the collaborative studies on development of WHO international HPV DNA standards and international quality assurance programs.

This helped laboratories to improve knowledge and skills of the various methods in details. The HPV LabNet built in these years is a very important project and it is necessary that this experience continues and grows further because monitoring the effectiveness of vaccination will begin shortly and it is very important to help the development of HPV laboratories in countries with lower resources and at the same time to facilitate sharing information and knowledge from experienced laboratories in industrialized countries.

P Abraham (Christian Medical College, India) presented the situation in India. In a country where over 130,000 women are diagnosed with cervical cancer, leading to over 74,000 deaths per year, this malignancy is a leading cause of death among middle aged women in India. A national cervical cancer screening program is yet to be implemented. The bulk of the HPV prevalence data in India is the result of specific funded studies conducted by health care and research institutions/agencies. Two HPV vaccine demonstration projects have been initiated and the International Agency on Research for Cancer (IARC) has started a Randomised Trial of Two versus Three Doses of HPV Vaccine across nine centers in India. The HPV vaccine has been recommended by the Indian Academy of Pediatrics Committee of Immunization for girls over the age of ten. MSD Pharmaceuticals (India), a subsidiary of Merck & Co. has been collaborating with Indian Council of Medical Research to bring the cervical cancer vaccine to the Indian public sectors at an affordable price. Right now, even the currently negotiated prices of both vaccines (Gardasil and Cervarix) are unaffordable for over 70% of target population in India. Further challenges with HPV vaccination are that the target group of girls is hard to reach and there may be cultural barriers to delivering this vaccine only to girls and as an anti-sexually transmitted infections (STI) vaccine. Logistic constraints of transportation and maintenance of the vaccine's cold chain are further concerns.

There is no national policy on quality assured HPV assays i.e. HPV DNA detection or pre/post vaccination screening. The WHO HPV RRL and other laboratories of similar capacity in the country need to network better. The development of a QA system and participation in proficiency testing of HPV DNA detection and genotyping and HPV serology is urgently needed in these laboratories. There is also a need to identify fully standardized and affordable assays for wider use in India. A suggestion put forward was that those in poor resource settings use assays such as *CareHPV* test (Qiagen) known for its quick turnaround time, accuracy, simplicity and robustness. This will certainly

facilitate the wider use of HPV DNA tests in cervical cancer screening. The Clinical Virology Laboratory at CMC Vellore, south India has conducted and continues to conduct HPV related studies that have been funded by the Department of Biotechnology and the Indian Council of Medical Research and has teamed up with US collaborators funded by National Institutes of Health. The laboratory has experience with the L1 based MY09/11 primers in a PCR-RFLP format, the Line Blot Assay using PGMY 09/11 primers (developed by Roche) and the Linear Array and has some experience with HPV VLP 16 and 18 ELISAs (VLPs provided by Professor Raphael Viscidi, Johns Hopkins, Baltimore). The laboratory looks forward to participating in an international proficiency testing program for HPV DNA and antibody detection and being part of the HPV LabNet. The setting up of the CHUV-RBH assay in this centre will be a definite step towards providing more cost-effective HPV testing methods in this region.

M Sinha (Kidwai Memorial Institute of Oncology, India) further presented the situation in India. The incidence of cervical cancer varies from 5.5 to over 30 per 100,000 women population. There is a lack of effective screening programs and a lack of awareness about cervical carcinoma among most Indian women which, in turn, has led to the lack of demand for screening and vaccination against cervical carcinoma. Recent studies and meta-analysis show that HPV type 16 and 18 cause almost 80% of cervical carcinoma in India. This implies that an HPV vaccine targeting types 16/18 is likely to offer substantial protection against cervical carcinoma in India. The other HPV types implicated in cervical cancer are types 45, 33, 35, 58, 59, 31. However, there are potential hurdles in implementation of the HPV vaccine. These include the prohibitively high cost of vaccine, conservative sociocultural beliefs, difficulties in reaching the target age group, incomplete coverage due to defaulters in multiple dose vaccine, cross-protection against other HPV types not included in the vaccine and whether booster doses will be needed. In India, vaccine with incomplete type coverage is a cause for concern due to lack of effective screening programs to detect premalignant lesions in vaccinated population. In addition in an immunized population, there is a possibility that infections with other HPV types may not be effectively removed by humoral immunity and also, a vaccine covering other high risk HPV types, introduced subsequently, may be ineffective due to "original antigenic sin". This is due to the inability of the immune system to mount an effective immune response to the later delivered vaccine genotypes due to the "locked in" immune response caused by the earlier genotype specific vaccine. All these issues

have to be kept in mind before implementing the HPV vaccination program in India. Alternative HPV vaccines that address at least some of these issues are the multivalent VLP vaccines, L2 based vaccines which have a broadly cross reactive coverage or the capsomere based vaccines which are easier to produce and maybe cheaper than the current vaccine.

Kidwai Memorial Institute of Oncology (KMIO) is a regional cancer centre in Bangalore catering to more than 10,000 new registrations every year. The department of Microbiology is involved in various HPV related projects in India. An HPV surveillance study was conducted in KMIO including the Microbiology, Pathology, Community Health and Social Work Departments. The study was on "high-risk HPV genotypes in cytology based screening program for cervical carcinoma in rural women in Karnataka, India: 2003-2006" (unpublished data). In this study campaigns were conducted in rural areas and urban slums and women were screened for cervical carcinoma by cytology. An in-house HPV genotyping method was developed to complement cytological screening, which was a Multiplex PCR-ELISA, based on PGMY 09/11 primers followed by Reverse Line Blot Assay.

Dr Sinha said that the "CHUV" assay learned in this workshop would be used in the various HPV related studies in the department. HPV serology would start later, though before a vaccination programme is implemented, since KMIO is the regional cancer centre in South India and needs to be equipped with the serology assay. This would be very important for looking for seroconversion among vaccinees and in the long run, uncovering the efficiency of any vaccination programmes.

J Ngomlac (Uganda Virus Research Institute, Uganda) presented the situation in Uganda. There is a population of 7.32 million women aged 15 years and older who are at risk of developing cervical cancer. It is further estimated that 2,429 women are diagnosed with cervical cancer every year. Though data are not available on HPV in the general population, in East Africa where Uganda is located, about 33.6% of women harbor cervical HPV infection.

PATH in close collaboration with the MoH, Uganda and other partners are implementing HPV vaccination in Uganda. It is being piloted in two districts of Nakasongola and Ibanda. There was massive sensitization which involved vaccine delivery, communication and advocacy strategies. The vaccine delivery strategy involved giving

vaccines as part of Child Days Plus. Vaccine was given to girls at school and efforts made to reach those outside school as well. Girls were selected by age and grade. The exercise involved National Immunization Unit at every stage of assessment and planning. The communication strategy was developed to disseminate accurate information to address low knowledge about cervical cancer. This was aimed at providing positive perception of vaccination regarding safety and side effects. Communication strategy also aimed at reaching out to decision makers. The advocacy strategy was to develop guidelines that set national standards for HPV Vaccination. It explained how HPV Vaccination is consistent with Uganda's health policy. Ten thousand girls of 10-12 years age have been vaccinated. A lot of demand for vaccine is realized. Two methods of monitoring are in place, which are school based and age based. In both cases there was need for good registry in place. The capacity of MoH determines the level of monitoring. A number of challenges were encountered that required sentinel sites to be defined. Restrictions included people movements (where refugees are involved), HPV detection methods in low resource setting, availability of testing facilities, timeline for follow-up and standardized testing protocols as well as algorithm. Laboratory activities to support HPV vaccination includes: proper cytology-histology screening program, cervical cancer screening and maintenance of HPV vaccine cold chain. Findings from this demonstration project were to serve as evidence for government to decide when and how to incorporate HPV vaccination into a comprehensive cervical cancer screening program.

MR Farzami (Reference Health Laboratory, Ministry of Health, Iran) presented the situation in Iran. Based on the data gathered by National Cancer Registry Program, cervical cancer has a low incidence and does not rank between the first ten most prevalent cancers in Iranian women. Pap smear screening method for cervical cancer prevention is well known. The MoH, Iran supports this program in various ways. It should be noted that the capacity for performing and reporting the Pap smear exists throughout the country. But the higher prevalence of other life threatening cancers (e.g. breast cancer) had caused cervical cancer to lose its place among the current health priorities.

Along with international efforts to reduce the burden of cancer, all attempts are targeted towards the preventable and/or treatable types. The established role of HPV in contributing to cervical cancer and recognized effectiveness of vaccination has made a solid platform for revising the current health priority setting. For this purpose a population based study has been designed by the Iranian Center for Disease Control and

Reference Health Laboratory (RHL), MoH to determine the prevalence of HPV and its types in Iranian Women.

The chance of participating in this comprehensive WHO HPV workshop had given a great opportunity for RHL not only to set up the "CHUV" assay but also to strengthen its core capacity. RHL will make use of this capacity to assess the national capability and also evaluate the feasibility of this method from a national point of view. Enrollment of RHL in HPV proficiency testing program, carried out by WHO, is a valuable situation that will enable the country to improve the validity of future achieved results and collaborate with other international reference laboratories. RHL will use all its capacities to team up with the HPV RRL in the Eastern Mediterranean Region and all other regional/national laboratories in order to form a network to standardize the techniques, share the experience, establish quality control and conduct population based studies. Incorporating HPV vaccine in the national expanded program on immunization should be in alignment with national health priorities and policies in future.

H Ihazmade (Institut National d'Hygiène, Ministère de la Santé, Morocco) presented the situation in Morocco. Cervical cancer is the most common cancer in women in Morocco. It presents a great public health problem both in incidence and mortality. The HPV vaccination is not implemented yet in the public health system in Morocco; however it is available in the private sector since 2005. There is no epidemiological surveillance data available and the implementation of HPV surveillance system is in process following a visit of WHO consultants and supported by Lalla Salma Association for Cancer Prevention and the MoH.

Staff and equipment are in place to support HPV work. Data management tools, as well as rapid communication between all partners are in place with feedback information to WHO Regional Office in Eastern Mediterranean Region (EMRO) and to WHO due to the experience of being a WHO national reference laboratory for polio, measles, influenza and rotavirus. SOPs are used for all activities in the laboratory, following national and international procedures. Therefore a sentinel surveillance of HPV and specimen management is planned.

A challenge to contributing to the international surveillance system of HPV and providing necessary information to evaluate HPV vaccination will be constrained due to the high price of HPV vaccination and testing. In the end the virology laboratory will

play an important role in vaccine preventable disease surveillance by providing useful information to guide vaccine implementation.

JA Basiletti (HPV National Reference Laboratory, PAHO RRL for the WHO HPV LabNet, National Institute of Infectious Diseases, Argentina) first presented the structure and function of the National Institute of Infectious Diseases in Buenos Aires, that belongs to the National MoH in Argentina. Currently this institute comprises four departments: Bacteriology, Parasitology, Mycology and Virology. Its main concern is the study of agents of medical interest from the perspective of pathogenesis, epidemiology and reference diagnosis. All laboratories are involved in national reference activities. At the Virology Department, international reference activities are on neuroviruses, respiratory viruses, hantavirus and HPV.

The Pan American Health Organization (PAHO) has a large and successful experience in immunization in the Americas. PAHO has Revolving Fund (RV) which is a mechanism for the purchase of vaccines. The RV is a bulk purchasing mechanism to make vaccines affordable for national immunization programmes. RV has been a significant catalyst for the fast, equitable and sustainable introduction of new and underused vaccines, and now HPV vaccines are being targeted. Panama is the only country that started universal HPV vaccination; Mexico has started but only in some regions. In all the other countries, it is only available in the private sector. In Argentina, the National Cervical Cancer Prevention Program is based on cytology; however it has a low coverage and unequal efficiency in the different provinces. Though HPV testing has not yet been incorporated in the national cervical cancer screening program, there is interest in including an HPV test to improve cytology. Although the MoH is not immediately introducing the HPV vaccine, HPV vaccination within a comprehensive cervical cancer program is being considered in the near future. HPV involves STI, oncology, immunization and epidemiology areas and it is a true challenge to coordinate an integrated work among them. The Reference Laboratory of MoH is in touch with these areas, trying to promote collaborative work. The field is being prepared for giving the laboratory support to vaccine introduction and monitoring. Argentine immunization policy is always in line with PAHO and follows PAHO guidelines.

The Argentine national reference laboratory took advantage of previous institutional experience in other laboratory networks to contact professionals from Argentine

provinces and identify regional issues. The Argentine national HPV laboratory network was launched five years ago headed by Mr Basiletti's laboratory. Its main aims are the diagnosis and prevention of HPV infection and related diseases, including the laboratory reference, research and the interaction and support of the National Programs of Epidemiologic Surveillance, Immunization and Cancer. Twelve out of 24 Argentine provinces are presently part of the network. The laboratory members feature different degrees of infrastructure, development and experience in HPV. Each province is autonomous regarding its decisions on health policies and the final decision lies in each jurisdiction. This depends largely on the budget, staff and infrastructure available. The development of collaborative projects is supported as needed, making the most advantage of the capacities of the different nodes involved in the network.

This laboratory is currently providing training through an active technology transfer, delivering QC panel, organizing workshops, and encouraging the interaction with public health authorities through collaboration in national immunization programmes, epidemiologic surveillance, and cancer prevention. In March 2009 the laboratory was selected, after expert review, as WHO HPV RRL for the Americas. Activities so far have been participation in HPV LabNet confirmatory testing and proficiency studies; implementing GP5+,6+ PCR-RLB and "CHUV" genotyping assays; moving on QA/QC programs (data loggers, SOPs, etc). This RRL participated in two proficiency panels for genotyping organized by the WHO HPV LabNet (the 2nd panel applying MY9,11 PCR-RFLP /GP5+,6+PCR-dot blot hybridization and the 3rd panel applying PGMY-RBH/GP-Reverse Line Blot systems). Data showed that both assays were proficient for most HPV types. This RRL also participated in the 2nd phase VLP-ELISA study, however the HPV serology is not routinely performed in this laboratory due to the lack of infrastructure for VLP production. Therefore this RRL would need the supply of qualified VLPs.

Future plan of this RRL includes: validation of genotyping assays recently set up in the laboratory, participating in the next WHO HPV LabNet proficiency studies and organizing the 1st PAHO/WHO Training Workshop on HPV Genotyping Laboratory Performance in 26-28 April, 2010, which is for participants from Latin American countries, who intend to be part of the Regional HPV Laboratory Network. It's anticipated that potential "national" HPV reference laboratories would be identified in this workshop and at least networking would be initiated to form a regional network. This RRL also plans to organize an HPV Laboratory Workshop for the Argentine Network in

the end of 2010 to update and discuss topics of common interest related to HPV surveillance and assess the HPV typing quality assurance program. Internships at the RRL will be offered to Latin American countries laboratory members and Argentine provinces, as well as site visits.

JE Rhee (Korea National Institute of Health, Republic of Korea) presented the situation in South Korea. Cervical cancer is the second most common cancer in women aged 15-44 years. Each year there are around 4,949 cases of cervical cancer and 1,327 deaths in South Korea. The incidence of invasive cervical cancer has decreased, while the incidence of cervical cancer in situ has increased from 1999 to 2005. It means that cervical cancer screening and vaccine implementation are important to prevent cervical cancer for public health aspect in South Korea. The prevalence of HPV infection and the predominant HPV genotypes, were investigated with Hybrid Capture 2 (HC2) and HPV DNA Chip, respectively, in women at high-risk. 194 of 417 high-risk women were HPV DNA positive by HC2 method and the prevalence of HPV infection was 47%. The prevalence decreased with age and the highest HPV prevalence was observed in women under 20 years of age. The proportion of women infected with high-risk HPV genotypes was much higher than that of women infected with low-risk genotypes and infected with both genotypes. In South Korea, the most prevalent HPV genotypes in high-risk women were HPV-16, -35, -51, -52, 58, and -18. HPV16 infected women were examined for HPV16 E6 and E7 intratypic variations. At the nucleotide level, 26 variants of the HPV16 E6 and E7 were identified including 12 silent mutations. At the amino acid level, the isolates showed 14 variants and the prevalent HPV16 E6 and E7 variants were E6 D25E, L83V, Q14H, I27R, H78Y and E7 N29S, the dominant being for E6 D25E and for E7 N29S. This could provide the baseline data to develop an area-specific therapeutic vaccine for cervical cancer in Asia region.

Two HPV vaccines were licensed by Korea Food and Drug Administration (KFDA). Those are Gardasil (licensed in June, 2007) and Cervarix (licensed in July, 2008). Post-licensure evaluation of the HPV vaccines needs are to determine the vaccine long-term efficacy and effectiveness, vaccination uptake and compliance, safety, and integration of vaccination with other strategies such as organized screening. However, it is hard to know how many women were vaccinated and who got the vaccine because HPV vaccine is not included in National Immunization Programme in South Korea. Accurate HPV DNA detection, genotyping and serology tests are necessary for obtaining precise

national data for HPV prevalence and genotype distribution in the evaluation of HPV vaccine implementation and monitoring of HPV vaccination impact. This training workshop enables experiencing the standardized HPV genotyping and serology tests practically and it would be helpful for HPV surveillance work in the future.

4. Conclusions

This training workshop provided comprehensive knowledge and information to the participants on all aspects about HPV laboratory testing, including laboratory performance of basic assays. The training program was contributed by the effort of the WHO HPV LabNet over the past years in the standardization and harmonization of HPV testing. This workshop also responded to the requests from countries for technical support on HPV laboratory testing, especially in developing countries where HPV laboratory activities are being set up. In particular, opportunities were given to the participants to present HPV laboratory activities in supporting HPV surveillance and vaccination monitoring in respective countries, which was very informative to promote communications and networking among countries and with the HPV LabNet in future work, e.g. strong interests from some participants were received in participating in the HPV LabNet proficiency study and also in participating in the HPV LabNet. Other critical issues were identified during the discussions in this workshop, e.g. high expense of HPV vaccines and limited implementation of vaccination in developing countries; high cost of commercial HPV assays; need for cheaper, standardized assays and supply of standardized critical reagents; need for further technical support; lack of strategic plan and funds for conducting HPV surveillance in some countries; etc.

A comprehensive HPV Laboratory Manual (developed by the WHO HPV LabNet) and critical materials were provided to the participants to take back to help them set up new assays in their laboratories. All relevant information, presentations in the workshop and useful references were provided to the participants in CDs. Participants regarded this training as very informative, helpful and timely as they are setting up the HPV laboratory testing capacity in their countries to support HPV epidemiological studies. Implementing good performance of HPV laboratory testing and international standardization will ensure that proficient and standardized assays are used in HPV epidemiological studies and reliable data are generated to promote international harmonization of HPV laboratory

testing and comparability of data across different laboratories. It is anticipated that participants will be able to assist the HPV LabNet Regional Reference Laboratories to provide technical support to other laboratories in the respective Region, when such need is identified.

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The final draft was prepared by **Dr TieQun Zhou**, QSS/IVB/FCH, WHO, Geneva, Switzerland; taking into account comments from the presenters and participants at the workshop.

Annex 1, 2 (attached)

Annex 1

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Annex 2

The 2010 WHO HPV LabNet Training Workshop on HPV Genotyping and HPV Serology Laboratory Performance

CHUV, Lausanne, Switzerland, 15-18 March, 2010

Agenda

Day 1, 15 March 2010

8.15-8.45	Registration	
8.45-9.00	Welcome address	D Nardelli
9.00-9.10	Self-introductions	Participants
9.10-9.20	Objectives and expected outcomes of the workshop	TQ Zhou
Session 1	General Knowledge and Information	
9.20-10.00	HPV biology, disease burden and vaccines	E Unger
10.00-10.30	Coffee break	
10.30-11.00	WHO activities in facilitating HPV Vaccine introduction	
	- WHO Biological standardization	
	- WHO HPV Laboratory Network	TQ Zhou
11:00-11.40	HPV testing options (DNA and serology)	
	- Strengths and limitations	E Unger
	Introduction of WHO HPV Laboratory Manual	E Unger
11.40-12.10	Monitoring HPV Vaccination impact - Why and how?	J Dillner
12.10-13.00	LUNCH	
Session 2	HPV Genotyping Assays	
13.00-13.30	HPV proficiency testing	J Dillner
13.30-14.45	Introduction of a non-commercial HPV genotyping assay evaluated by WHO HPV LabNet: PGMY-RBH assay	
	- including quality control aspects, DNA extraction and introduction to practical work	
		R Sahli

10.15-11.00	<i>Laboratory practice: ELISA</i> <i>- Introduction and blocking plates</i>	D Nardelli
11.00-11.45	Quality control of VLPs and pseudovirions	J Dillner
11.45-12.30	<i>Laboratory practice: ELISA</i> <i>- Samples</i>	D Nardelli
12.30-13.30	LUNCH	
13.30-14.30	<i>Neutralization assay</i> <i>-Demonstration and practical aspects (I)</i>	D Nardelli
14.30-14.45	<i>Laboratory practice: ELISA</i> <i>- Ssecondary antibody</i>	
14.45-15.45	<i>Neutralization assay</i> <i>-Demonstration and practical aspects (II)</i>	D Nardelli
15.45-16.00	<i>Laboratory practice: ELISA</i> <i>- Detection, substrate</i>	D Nardelli
16.00-16.30	Coffee break	
16.30-17.30	<i>Laboratory practice: ELISA</i> <i>- Calculations and discussion</i>	D Nardelli
17.30	Trouble-shooting & Discussion Closure of day 3	

Day 4, 18 March 2010

Session 4	International Standards, Quality Control and Quality Assurance	
8.30-9.00	Laboratory Quality Control - Why and How?	E Unger
9.00-9.30	International Standards/ Reference Reagents	M Ferguson
9.30-10.00	Useful tools of quality assurance of HPV testing in HPV LabNet <i>- Proficiency testing and Confirmatory testing</i>	J Dillner
10.00-10.30	Coffee break	
10.30-11.00	Assay validation	M Ferguson
11.00-11.30	Secondary standards- preparation and use	M Ferguson
11.30-12.00	Critical issues and quality control of specimen collection for HPV testing	E Unger
12.00-13.30	LUNCH	

13.30-14.00	Data management- why and how?	J Dillner
Session 5	Presentations from participants (15 min each)	
14.00-	Each participant gives an introduction on following issues in individual country:	
	- HPV vaccine implementation	
	- HPV surveillance and vaccination impact monitoring	
	- Lab activities in supporting above work	
	- Perspectives: future workplan after this training course	
-17.00	Discussions, Conclusions	
	Closure of meeting	