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## **Meeting Report**

# **WHO meeting on the standardization of HPV assays and the role of WHO HPV LabNet in supporting vaccine introduction**

**WHO/HQ, Geneva, Switzerland**

**23-25 January 2008**



## **EXECUTIVE SUMMARY**

In anticipation of the implementation of new prophylactic HPV vaccines, the WHO is supporting the establishment of a global HPV laboratory network (LabNet) whose mission is to “contribute to improving the quality of laboratory services for effective surveillance and monitoring of HPV vaccination impact through enhanced, state-of-the-art laboratory support”. To date, two Global Reference Laboratories and seven Regional Reference Laboratories have been assigned to the WHO HPV LabNet.

WHO convened a meeting at its headquarters in Geneva, 23-25 January 2008 to review the activities of the WHO HPV LabNet in its first year and to plan for the next step of the WHO HPV LabNet in order to meet the future demands of vaccination programmes. The WHO meeting placed particular emphasis on the harmonization of WHO HPV LabNet practices and standardization of HPV assays as these are crucial for the success of the WHO HPV LabNet in conducting studies measuring HPV disease burden and vaccine impact.

The first day of the meeting was reserved for addressing issues specific to the WHO HPV LabNet and was attended by all members of the network as well as representatives of WHO Headquarters, Regional Offices and Collaborating Centres involved in HPV-related work. The objectives were to review the activities of the LabNet over the past year; identify current capacity and propose future work for each Reference Laboratory and the LabNet as a whole; address critical issues and gaps within the LabNet and promote communications between LabNet members and with the WHO Regions. The first day of the meeting was concluded with WHO HPV LabNet members proposing revisions to the generic Terms of Reference for 2008-2009. The report of this WHO HPV LabNet meeting is available on the HPV LabNet SharePoint located on the WHO website.

The objectives of the second and third day of the meeting (24-25 January 2008) were to assist in developing the workplan for the WHO HPV LabNet so that it functions efficiently and prioritizes its activities effectively; identifying gaps and prioritizing tasks. It was therefore appropriate for WHO to bring together the LabNet members, experts from additional HPV laboratories around the world, representatives of national regulatory and control authorities, non-profit organizations and industry. Critical issues included:

- Presenting WHO strategic programmes in supporting HPV vaccine introduction

- Reviewing the current status of standardization in HPV testing and experiences from industry about laboratory methodologies for clinical evaluation of HPV vaccines
- Reviewing progress in development of International Standards and identifying potential needs for additional standards and reference reagents
- Discussing ways of building the capacity of the global WHO HPV LabNet
- Seeking perspectives from the WHO Regions and the other organizations about the role and function of the LabNet in supporting HPV vaccine implementation
- Reviewing a draft Global HPV Laboratory Manual

Upon the conclusion of the meeting in a closed session, recommendations were identified to assist the LabNet in fulfilling its mission. These included recommendations on reinforcing the WHO HPV LabNet, on HPV assays and standardization and to WHO and public health authorities in supporting the WHO HPV LabNet.

## **Background**

The WHO global Human Papillomavirus (HPV) laboratory network (LabNet) was established in 2006 with the mission to contribute to improving the quality of laboratory services for effective surveillance and monitoring of HPV vaccination impact through enhanced, state-of-the-art laboratory support. WHO initiated these activities as part of a Bill and Melinda Gates Foundation-funded project, contributed by a group of collaborating institutions working in partnership, which includes PATH (Program for Appropriate Technology in Health) in Seattle, Harvard University in Boston, IARC (International Agency for Research on Cancer) in Lyon, ICO (Institut Català d'Oncologia) in Barcelona and WHO in Geneva

([http://www.who.int/biologicals/areas/human\\_papillomavirus/WHO HPV LabNet/en/index.html](http://www.who.int/biologicals/areas/human_papillomavirus/WHO_HP_V_LabNet/en/index.html)).

## **Opening remarks**

**Dr D Wood**, coordinator of the Quality, Safety and Standards (QSS) team of the Department of Immunization, Vaccines and Biologicals (IVB), welcomed participants to the meeting. He informed participants that the WHO Executive Board was meeting currently in Geneva. This is a subset of 22 of the 193 countries who meet to discuss the agenda of WHO in detail. The

WHO–UNICEF Global Immunization Vision and Strategy (GIVS), which was developed to give the organization guidance for the next 10 years, was discussed. There is universal support for immunization programmes but the opportunities and risks have to be considered as new vaccines are developed and introduced. Countries have indicated that they are looking to WHO to facilitate them in the decision-making process including risk-benefit analysis and cost-effectiveness of immunization programmes. This HPV working group fits into the call to generate evidence to facilitate decisions on HPV vaccines. The mission of the WHO HPV LabNet is to promote international comparability and quality of laboratory data. WHO initiated these activities as part of a Bill and Melinda Gates Foundation-funded project, contributed by a group of collaborating institutions that includes PATH (Program for Appropriate Technology in Health) in Seattle; Harvard University in Boston; IARC (International Agency for Research on Cancer) in Lyon; ICO (Institut Català d'Oncologia) in Barcelona and WHO in Geneva.

Dr Joakim Dillner agreed to Chair the meeting and Morag Ferguson to serve as Rapporteur.

Dr TQ Zhou reminded the group of the objectives of the meeting which were to assist in developing the workplan for the WHO HPV LabNet so that it functions efficiently and prioritizes its activities effectively. The LabNet also needs to identify gaps and to prioritize tasks. It was therefore appropriate for WHO to bring together representatives from the WHO Regions, stakeholders and HPV experts.

## **Session 1 WHO strategic program in supporting HPV vaccine introduction**

**Dr Wood** described the work on HPV in the broader picture of the work of WHO on norms and standards which is a core activity of WHO. WHO is committed to support the development of relevant and credible evidence to inform decisions on introduction of new vaccines and tools to monitor the effectiveness of new vaccines. In addition to ensuring that 100% of vaccines used in all national immunization programmes are of assured quality, the goals of GIVS are to increase and sustain vaccine coverage, reduce morbidity and mortality of vaccine preventable diseases, facilitate the development of new vaccines and strengthen surveillance systems. The WHO strategic approach to vaccines covers all stages from research to disease prevention with key teams of the WHO Initiative for Vaccine Research (IVR), Quality, Safety and Standards (QSS) and Expanded Programme on Immunization (EPI). Within QSS, there are groups dealing with norms and standards; regulatory processes

and quality of vaccine production, supply and financing; vaccine delivery and accelerated programmes; immunization safety, monitoring and disease burden assessment and the development of new vaccines. There are three types of norms and standards for quality, safety and efficacy of biological products and technologies: 1) global written standards which provide guidelines on the production and quality control of vaccines and biological and are published in the WHO Technical Report Series; 2) global measurement standards and reagents used in the validation and control of assays and 3) support for the scientific evidence base for global standards. The Expert Committee on Biological Standardization (ECBS) is mandated by the constitution of WHO and reports to the Executive Board which advises the Director General. A large number of other committees exist, and another important group is the Strategic Advisory Group of Experts (SAGE) which advises the Director of IVB and the Director General. SAGE will decide whether to advise on universal immunization for HPV. The development of standards for HPV has to compete with other priorities when it comes to developing International Standards (IS) and it is important that decision-makers such as ECBS and SAGE are well-informed of the requirements of the WHO HPV LabNet. In relation to vaccine regulation, countries fall into three groups: 1) countries whose vaccines for national immunization programs are provided via UN agencies; 2) procuring countries that purchase vaccines for their own use. Many of these countries, however, do not have appropriate expertise to ensure that they are purchasing vaccine of good quality and 3) vaccine-producing countries. Written guidelines for HPV vaccines were approved in 2006 (1) and these cover quality control as well as non-clinical and clinical evaluation. These guidelines will be used by national pharmacopoeias, manufacturers and product users, as well as in the pre-qualification of vaccines for supply to UN agencies. If vaccines are to be of assured quality, then the National Regulatory Authority (NRA) in the country of production must be independent from the vaccine manufacturers. Furthermore, the NRA must be fully functional with respect to a series of WHO-established indicators [2, 3].

Dr Wood outlined the activities of the Global Framework for Immunization Monitoring and Surveillance (GFIMS) which pulls together work on immunization and surveillance. The activities of the WHO HPV LabNet fit into the goals and objectives of this organization through its activities on building surveillance capacity at country level for disease-burden estimates and impact monitoring in preparation for new or recently introduced vaccines. Several new vaccines are becoming available and the WHO HPV LabNet will have an important role in providing disease surveillance data to national public health authorities

making decisions on priorities. To justify the cost of the vaccine the WHO HPV LabNet may also be expected to provide data on the impact of vaccination programmes.

**Dr K. Irwin** (Initiative for Vaccine Research [IVR]/IVB, WHO) outlined WHO's strategic plan in facilitating HPV vaccine development and introduction. As yet there are no WHO recommendations on use of HPV vaccination in national immunization programmes. WHO's current position is that countries should make decisions about HPV vaccine introduction according to several criteria including national health priorities; scientific evidence of the burden of HPV-related disease; HPV type distribution in cervical cancer and pre-cancer cases in relation to vaccine-related HPV types; the level of infrastructure for delivering a three-dose primary vaccine series to young adolescent females; cost-effectiveness and affordability; and capabilities and resources for vaccine monitoring. A range of committees influence policy on HPV vaccines and there is therefore a complex process of interactions regarding the advice given and the recommendations ultimately made by WHO at the advice of the immunization Strategic Advisory Group of Experts (SAGE). Following any decision to recommend implementation of HPV vaccination programs, a range of organizations may respond with decisions about vaccine financing and procurement for middle and low income countries. In November 2007, SAGE stressed that clinical trials demonstrate that vaccine introduction would greatly benefit developing countries with high cervical cancer mortality and limited screening, as well as countries with established screening programmes. However, developing delivery programs for young adolescent females will be challenging in most countries because routine vaccination through any method is rare in this age group. SAGE also emphasized a need to develop strategies to monitor vaccine impact and safety after introduction, especially in low and middle income countries. SAGE also advised the WHO Human Papillomavirus Expert Advisory Group (HEAG) (now renamed as the WHO HPV Vaccine Advisory Committee), to address high priority issues about vaccine safety, efficacy and delivery that can facilitate the decision-making process for vaccine introduction as well as advise WHO in supporting the monitoring of vaccine safety and the impact of HPV vaccination programmes, where the WHO HPV LabNet has a role. Monitoring HPV vaccine impact will differ from other vaccines because the target age group is outside EPI programmes that reach infants and young children. Additional factors that make HPV vaccine monitoring unique from other vaccines are the lack of surveillance capacity for HPV-related disease in most countries and the long latent period between HPV exposure and development

of the most serious vaccine- preventable HPV-related disease. For example, it may take more than 10 years to develop cervical cancer after initial HPV exposure.

Using HPV tests as vaccine monitoring tools is hindered by several issues: 1) The very high efficacy of HPV vaccines in clinical trials means that the minimum level of protective antibody conferred by vaccination is not yet known. 2) Tests for HPV DNA are expensive and tests for anti-HPV antibodies are neither very sensitive nor well standardized. Some tests involve pelvic examination which has cultural implications in some countries. 3) The majority of HPV infections are benign and transient and persistent infection can only be determined through repeat testing over time. Nevertheless, HEAG recommended in September 2007 that a lack of monitoring capacity should not delay vaccine introduction. Alternative methods of assessment should be considered. For example, in order to determine the minimum level of protective antibody, the need for boosters or alternative dosing schedules in developing countries, antibody levels may be assessed through clinical trial participants, routine or *ad hoc* HPV serosurveys. HPV DNA testing can be used to monitor prevalence or incidence of vaccine-related types but requires careful design because of wide variation in infection by age and difficulty of sampling women through pelvic examinations in some countries. Monitoring vaccine impact through cytologic or histologic outcomes is more clinically relevant but would require new linkages with vaccine registries. Such monitoring may be biased in settings without organized, high-coverage screening programmes, and may not be feasible in many low and middle income countries. In September 2007, HEAG recommended minimal monitoring requirements, such as vaccine coverage surveys and passive, post-marketing safety systems, for low and middle income countries.

SAGE is likely to discuss recommendations on HPV vaccines in 2008 which will include considerations for monitoring vaccine impact and safety after vaccine introduction and for determining to what degree these monitoring systems must be in place before implementation. As of January 2008, Gardasil® was licensed in 90 countries and Cervarix® in 52 countries. Countries have licensed HPV vaccine for use with different target populations. Most countries have licensed HPV vaccine for use with girls and young women, often between the ages of 10-25 years. Some countries however have licensed Gardasil® for use with boys and young men and Cervarix® for use in woman up to 55 years of age. HPV vaccines are also recommended for use in national immunization programmes in several North American and Western European countries, some of which have called for monitoring

after vaccine introduction. WHO regional workshops on cervical cancer prevention and HPV vaccines have taken place in WHO Regional Offices for Europe (EURO), South-East Asia (SEARO) and Western Pacific Region (WPRO). Workshops in the remaining WHO Regions will take place in 2008. All meetings have emphasized the need for and the value of HPV testing in determining which HPV types are associated with HPV-related disease and how this might facilitate decisions on vaccine introduction. Regional meetings also stress the need for monitoring vaccine safety and impact as such systems currently do not exist in many countries. The WHO vaccine prequalification process will ensure that vaccines of high quality are made available to countries whose vaccines are procured by UN agencies.

Dr Dillner commented that the description of the context of the WHO HPV LabNet work was essential and the group agreed that the work of the WHO HPV LabNet is crucial to increase capacity to monitor HPV vaccine impact. Dr D.A. Galloway (Fred Hutchinson Cancer Research Center, USA) commented that after the introduction of HPV vaccines, industry and research communities will have a role in post-marketing surveillance and queried how the WHO HPV LabNet would interact with these programmes. As there are limited staff at WHO HQ and Regional Offices, input from experts around the world will be needed. Interactions are already ongoing with IARC who is monitoring HPV types and links with cervical cancer. Dr S. Garland (Regional WHO HPV RL, Royal Women's Hospital, Australia) indicated that it is important that monitoring is done well but that not all laboratories can do it as specific expertise is needed. Some countries with testing capacity should perform post-vaccine monitoring to serve as models for and be representative of other countries, so that not all countries will have to implement these systems. Dr E.R. Unger (Global WHO HPV RL, CDC, USA) commented that implementation of vaccine may also result in improved screening for cervical cancer so studies will not be able to determine if declines in HPV-related disease are due to vaccination, screening, or both.

The policy for vaccination of young boys/men was also raised and it was reported that SAGE will consider this.

**Dr TQ Zhou** then gave an update on the WHO global HPV LabNet including its history of development and the network's Terms of References (TORs). This project is currently funded by the Bill and Melinda Gates Foundation and is aimed at harmonizing and standardizing HPV laboratory testing procedures. This network will support consistent laboratory evaluation of regional disease burden and monitor the performance of HPV vaccines so that countries are prepared when HPV vaccines are introduced. This project also included the

creation of the WHO/ICO Information Centre on HPV and Cervical Cancer to facilitate global, regional and, country-specific decisions on current and novel options for cervical cancer prevention.

The participants of a WHO technical workshop held in August 2005 agreed that an HPV laboratory network should be established with Global, Regional and National Reference Laboratories [4]. A call for applications went out in April 2006 followed by a selection process that involved external expert review and a site visit of prospective applicants. So far two Global and seven Regional Reference Laboratories have been appointed and the National Institute for Biological Standards and Control (NIBSC) in South Mimms, UK, collaborates with the WHO HPV LabNet in standardization projects. Each laboratory had an agreed TOR which covered the provision of scientific and technical advice, quality assurance, training and communication. Each laboratory was also provided some financial support as seed funds for use towards building testing capacity.

An International Reference Reagent for anti-HPV type 16 serum was approved by ECBS in October 2007. Proposals for the establishment of International Standard (IS) for HPV type 16 and 18 DNA will be considered at the next ECBS in October 2008. Most of the WHO HPV LabNet members participated in the collaborative studies to assess the suitability of these materials. A proficiency study, co-ordinated by Dr Dillner, to evaluate HPV DNA testing capacity has also been undertaken.

The WHO HPV LabNet members met in Lausanne in June 2007 for a training session and it is hoped that these laboratories will provide training and technical support to other laboratories and WHO. Progress has been made in the development of a laboratory manual which will give guidance on the performance and Quality Assurance (QA) of HPV laboratory testing. The first edition of the LabNet newsletter was developed in October 2007 and broadly distributed. A SharePoint workstation for WHO HPV LabNet members has been set up at WHO to provide relevant documents to promote information sharing and a WHO public website for the WHO HPV LabNet was launched in November 2007 which has links to other WHO sites dealing with HPV. Under agreement with the laboratories, an independent expert review of the LabNet annual reports has been conducted in order to evaluate the progress of the WHO HPV LabNet and to identify gaps in each laboratory as well as in LabNet overall activities. Two external experts reviewed the reports and the review comments and summary report were communicated to individual Reference Laboratories. Critical points from the

experts' review were: 1) Achievements during the 1<sup>st</sup> year of the WHO HPV LabNet are considerable. 2) The majority of the Reference Laboratories have fulfilled requirements specified in their respective Terms of Reference (TOR). 3) Several aspects specified in the general TOR could not be fulfilled during the 1<sup>st</sup> year as an initial phase was required for establishing the WHO HPV LabNet. 4) In current TORs, the statement of "upon request" might be seen as a passive request which might be taken as low priority, as mirrored in some reports. The provision of well defined programs may be advisable for future TORs. 5) HPV DNA proficiency study was very helpful in identifying gaps so that LabNet members can make further improvements. 6) Confirmatory testing is an extremely important aspect for QA. This should be considered as a mandatory requirement. 7) Training at the Regional level should be emphasized during the forthcoming period. 8) Active support from WHO may be necessary to facilitate communication with public authorities. 9) WHO recommendations on how HPV infection and vaccination should be monitored are anticipated. 10) The capacity for serving as a Regional Reference Laboratory in some labs should be strengthened. WHO shall conduct site-visits or re-evaluations and provide further guidance and support. 11) With efforts from the WHO HPV LabNet and WHO Regional Offices, it should be ensured that the LabNet activities are initiated, and that the countries are aware of LabNet activities, in particular in countries who introduce vaccination programs.

In conclusion, the laboratories are operating at different levels, there is a lack of standardization with regard to laboratory testing and more funding is required. During 2008 the work of the LabNet at the Regional level shall be reinforced. Expansion of the LabNet to include laboratories at the National level will be considered in due course.

## **Session 2 Current status in HPV testing and standardization**

**Dr E.-M de Villiers** (DKFZ, Germany) reviewed critical standardization issues in HPV DNA assays and addressed the issues that arise at the bench and not at a political or organizational level. The WHO HPV LabNet is different from other laboratory networks as it comprises both research and diagnostic laboratories; whereas other networks are mainly diagnostic laboratories and national public health laboratories. The aims of HPV DNA testing (single versus multiple types, which HPV types to test for, etc) must be decided at the onset for each individual laboratory as these will vary and results can only be interpreted within the framework and limitations of the applied test. The aim of any HPV DNA test is usually to identify either multiple or single HPV types and/or to distinguish between high- and low-risk

HPV types. The validity of the results from a laboratory depends greatly on and reflects the basic principles of laboratory practice, sample handling, the kits or reagents used, the inclusion of controls and the interpretation of results. The minute details of the basic principles that are critical and important in ensuring assay validity are very often not mentioned during training courses as they are often taken for granted. These include ensuring that all assays are performed under controlled conditions through, for example, the calibration of pipettes; ensuring that freezers and refrigerators are kept at the correct temperature (e.g. doors not being opened frequently or for prolonged periods of time) in order to ensure the stability of reagents; and aliquoting reagents to avoid contamination. Separate locations are required for different stages of the assay. Although it may be difficult to compare the performance of different batches of assay kit or reagent, consideration should be given as to how this can be achieved most easily, for example, through inclusion and monitoring of relevant controls. There are pros and cons for the use of various materials as controls: 1) Clinical samples are not practical because of variability between batches. 2) Cell lines are not optimal for specific HPV types as the copy number of SiHa and HeLa cells is not agreed. 3) Placenta DNA as a negative control is not advised as the quality of commercially available samples vary. 4) Independent controls are required in addition to the controls supplied with kits as these may also be variable. 5) The use of several monovalent HPV types or mixed HPV DNA standards should be considered. All HPV types cannot be included as individual positive controls, but at least those which are known to occur frequently in the respective population, e.g. where 52 and 58 are more prevalent, they should be run as single standards. The interpretation of results is also crucial, particularly if any result is the same as the HPV type present in the control. In such instances, cross-contamination should be considered and the samples re-tested using original sample DNA and including a different HPV type as control. The determination of an appropriate cut-off in quantitative and qualitative tests is also important. Participation in external assessment is mandatory. Dr de Villiers also highlighted the need to include detailed instructions for opening ampoules of the candidate HPV DNA International standards as they contain high copy number plasmid which could result in contamination of a working area.

Dr de Villiers also recommended that training be undertaken in the trainee's own laboratory in order for the trainer to observe working conditions and standardization under the given laboratory conditions and practices. Other participants indicated that they have had good experience in training in a reference laboratory.

It was proposed that basic concepts such as precautions on the bench be included in the HPV laboratory manual. The target sensitivity of the DNA tests was suggested to be 500 copies.

There was general agreement that HPV Reference Laboratories must have the capacity to test for a large number of different HPV types. As a component of monitoring vaccine impact, reference laboratories need to be able to identify both cross-protection and HPV type replacement. This requires the ability to perform HPV DNA testing for many different types.

Also it was indicated that, although high levels of virus is predictive of disease, following vaccination there may be a need for high analytical sensitivity to demonstrate that virus is not present. More sensitive HPV DNA tests may therefore be needed. It was however strongly argued against the necessity of quantitative PCR analyses.

**Dr M. Ferguson** (NIBSC, UK) then reviewed critical standardization issues in HPV serology assays. Neutralizing antibodies are probably the primary mediator of this protection and the WHO Guidelines for HPV vaccines [1] suggest that assays to measure neutralization potential are used initially for assessing the antibodies induced by the HPV vaccines. Once the neutralizing antibody response has been well characterized, the use of alternative assay methods, such as Enzyme Immunoassay (EIA), may be proposed as long as it is supported by a detailed analysis of the correlation with the neutralization test. *In-vitro* neutralization tests involving pseudovirions (PsV) carrying a marker plasmid to easily score infection are complex and labour-intensive. Such assays have recently been adapted to high throughput testing and recent improvements have resulted in an increase in the analytical sensitivity so that they may now be as sensitive as EIAs with respect to end-point dilutions. In the second WHO collaborative study the starting dilution used by each laboratory varied but the candidate standard was scored positive in all assays in four of the laboratories. A range of enzyme immunoassays were used, namely, direct EIAs, capture EIAs and competitive EIAs. Of the enzyme immunoassays used in the two WHO studies, VLPs were from different sources, produced by several different methods. The majority of the assays undertaken by participants were direct EIAs, involving coating EIA plates with VLPs. In the first study in 2003, some participants did not score the negative as negative in assays of some serotypes and some participants reported naturally immune sera as reactive for the wrong serotype. However, when NIBSC recalculated the crude readout data in relation to the value of a simultaneously analysed standard serum, results were remarkably consistent between laboratories. This argues that the main priority for improving serology is to ensure that results are always reported relative to a working standard which is traceable to the WHO Reference

Reagent which would facilitate the identification of level of reactivity indicative of positivity. Sera from vaccinees were scored as reactive (although at low titres) in assays for antibodies to other types.

Critical QA issues which need to be considered are: 1) use of an international reference reagent as a standard for calibrating working standards; 2) reporting of results in internationally agreed Units; 3) identifying a universally agreed cut-off level for sero-conversion (defined in Units); 4) sourcing and quality control of VLPs; 5) control of specificity and 6) inclusion of controls on every plate or assay run.

Conformationally intact VLPs of good quality are essential for adequate performance of Enzyme-Linked Immunosorbant Assays (ELISA). The quality of VLPs may be determined through a number of tests such as their reactivity with monoclonal antibodies with neutralizing activity, electron microscopy, etc. The performance of each new batch of VLP needs to be controlled for specificity through testing serum samples of known reactivity from HPV DNA positive patients as positive controls and serum from HPV DNA negative virginal women or from children 2-10 years of age as negative controls. Disrupted HPV VLPs or bovine papillomavirus (BPV) VLP may be used as a negative control on separately coated plates. In the WHO studies the sensitivity of assays varied and a wide range of starting dilutions were used by different laboratories, in that negative results from different laboratories were reported as <6, <8, <10, <20, <50 and <100, emphasizing the need to use international standards. The design of assays must also be considered and it is preferable that replicates are made from independently repeated dilutions of freshly made solutions and not from sub-sampling from a single dilution series as this could result in underestimation of the within-assay variability. The order of samples on a microtitre plate may lead to a consistent bias in the relative potency estimates obtained. The controls which should be included on every plate or assay run are: a negative control; a standard against which unitage is determined and which should be traceable to the WHO reference reagent; and a sample of known reactivity so that performance of the assay is known. The reactivity of the standard may also indicate this. The determination of the cut-off for sero-conversion is important. This may be based on the response of the negative control standard sera, but should preferably be internationally agreed. The expression of results by various laboratories also varies and should be relative to an international reference reagent standard and be expressed in units.

During the discussions it was agreed that the assignment of a cut-off value is very important as is standardization of assays through use of appropriate international reference standard sera. Validation of any proposed assays should be performed.

**Dr D. Galloway** (Fred Hutchinson Cancer Research Center, USA) described studies on the characterization of the HPV antibody response and emphasized that HPV seropositivity cannot be a gold standard for assessing infection or long-term response to vaccination. There is still limited information on epitopes recognized by HPV antibodies, what they are and whether they are the same following natural infection and vaccination. Most immunoassays are based on binding of VLPs either directly to a plate or to a monoclonal antibody on a plate, and such assays require good VLPs. Separate plates are required for each serotype. However, multiplex assays have the advantage in that they limit the use of antigens, are easy to perform, and multiple types can be assayed at the same time. In competitive EIAs or competitive Luminex assays, human sera compete with HPV-specific monoclonal antibodies. A potential pitfall of this approach is that one may get steric hindrance and therefore a misleading result. In neutralization assays, a 50% reduction in colour is usually indicative of positivity. Approximately 50-75% of HPV DNA positive individuals are reactive in antibody assays. Antibody responses to Gardasil® give titres 1-2 logs higher than in natural infection for HPV-16 which then plateau at levels less than a log higher than natural infection. For the other vaccine HPV types, the responses to vaccines plateau to levels found in naturally infected women. In order to look at cross-reactivity, a sensitive assay is needed. Information from crystallisation studies has given insight into the epitopes recognized by antibodies and monoclonal antibodies with neutralizing activity binding to surface exposed loops. Competitive neutralization assays, in which each of the HPV 16 L1 loops were mutated singly or in combination to the homologous HPV 31 L1 loops, identified loops that commonly reacted with human sera. The HPV 16 neutralizing monoclonal antibodies that were tested blocked interaction with the Extracellular Matrix (ECM) or Glycosamino-glycans (GAG).

It was reported during discussion that the cost of a Luminex assay for a single serum sample is approximately \$2 US so it is cost effective to test thousands of sera. However, the equipment for Luminex may cost \$50,000 -60,000 and needs good maintenance and skilled staff. The usefulness of chimeric VLPs for assessing antibody response was raised, but it was felt that it was not well known whether chimeric VLPs folded into the same conformation as

wild type VLPs. The Luminex assays were considered useful for doing comparative assays, but are not a definite substitute for neutralization tests.

**Dr L.J. van Doorn** (DDL Diagnostic Laboratory, The Netherlands) described various aspects which must be considered for HPV DNA testing. Assays must be of appropriate sensitivity and a range of viral loads should be able to be detected by different assays as there is a difference between analytical and clinical sensitivity and levels of HPV DNA detected at different stages of disease. Different primer sets result in broad spectrum or type-specific detection. For assays detecting single genotypes there is no competition in detection, whereas there is competition in assays in which multiple types are present. Some assays such as the SPF10 assay detect more than 50 different HPV types. Positive samples identified by the SPF10 assay can then be genotyped by using the reverse hybridization line probe assay (LiPA), which identifies 25 genotypes. For all PCR assays, sample heterogeneity may result in false-negative PCR results, especially in samples with low viral load. The use of any single broad-spectrum test to detect HPV genotypes will underestimate the prevalence of certain genotypes, due to competition between genotypes in mixed infections. Laboratories should document their testing algorithms. The same algorithms can be used for swabs and biopsies. In a clinical study, 1113 samples were tested following an HPV testing algorithm developed at Delft Diagnostic Laboratory (DDL). Seventy of the 74 samples which were positive for HPV-16 contained multiple genotypes. For samples with low viral load, of 47 samples initially scored negative, 19 were found to contain multiple genotypes. It was concluded that this was due to sample heterogeneity and competition between different genotypes in mixed infections. Biopsy specimens are heterogenous, varying in size and method of fixation. DDL therefore tests specimens by a sandwich approach whereby the outside sections of a biopsy sample are examined by hematoxylin and eosin staining and the inner section tested by PCR. This process is contamination prone and knives need to be changed but this approach can link pathology to detection. The combination of broad-spectrum SPF<sub>10</sub>-PCR followed by LiPA and HPV-16/-18 type-specific PCR prevents false-negative results due to PCR competition in samples with multiple infections [5].

Dr van Doorn emphasized that validation of all aspects of HPV testing is required. The International Conference on Harmonization (ICH) provides guidelines for validating analytical procedures. The validation criteria addressed at DDL include: sensitivity/limit of detection (LOD); specificity (including HPV subtypes and variants); accuracy; reproducibility (e.g. within and between different kit lots or between different laboratory

technicians); robustness (e.g. the capacity of PCR and LiPA to remain unaffected by deliberate variations in the procedure); and interference (e.g. assessing PCR inhibition of samples collected on swabs, stored in PreservCyt and extracted by MagNAPure ). At DDL, all reagents used in in-house assays are produced under ISO13485 certified conditions as required for *in vitro* diagnostic tests in the European Union. Dr van Doorn emphasized that consistency is required across all phases of a study and even though it is tempting to adapt assays and make changes over time, this would not facilitate comparison of results. Sampling is also critical as low-quality sampling and inadequate sample handling cannot be compensated by high-quality testing. The entire process from sampling to data management and reporting needs to be controlled.

**Dr D. Sikkema** (Merck Research Laboratories, USA) indicated that because of the clinical efficacy of the vaccines, there is, as yet, no recognized immune correlate for protection for HPV and cervical cancer. He discussed the persistence of vaccine-induced antibodies to HPV which has now been demonstrated beyond 5 years and immune memory demonstrated by a rapid, ~100-fold boost in titre following an antigen challenge at month 60. He emphasized that characteristics of immune correlates are not just quantity of antibody induced, and may include antibody avidity, isotype, subclass, or memory responses, and reminded participants that the Food and Drug Administration (FDA) defines a correlate of protection as "a laboratory parameter that has been shown from adequate and well-controlled trials to be associated with protection from clinical disease." Dr Sikkema discussed the merits of two different immunoassay methods to measure antibodies to HPV and stated that they are validated in accordance with ICH guidelines. One is the Merck competitive assay for antibodies which is based on the ability of sera to compete for VLP binding with type-specific neutralizing antibodies to a single conformational epitope [6]. This assay provided the primary immunological endpoint measurements in support of Merck's vaccine product. Dr Sikkema stated that this is the only immunoassay designed to measure type-specific antibody responses, something that an anti-VLP immunoassay cannot claim. The second method is a direct binding assay that measures IgG antibody against the entire VLP. This assay can be used to measure IgA, IgM, or IgG subclass antibodies and may also measure antibodies directed against related, non-vaccine types. A reference standard is included in all assays and must be suitable to support long-term studies. For HPV, neutralizing antibody titres inversely correlate with the detection of HPV 11 tumors in a mouse xenograft system (a

true neutralization model). Dr Sikkema stated that Merck's assays correlate well with the pseudovirion-based neutralization assay methods.

The HPV Multiplex PCR assay used by Merck targets three genes (L1, E6, and E7) which results in increased sensitivity and specificity and increases the likelihood of detecting any HPV genetic variants or integrated forms. Each Multiplex PCR assay is type-specific (eliminating cross-reactivity and competition) with limit of detectability of <10 copies. The methodology for the isolation of DNA from clinical specimens for the WHO HPV LabNet needs to be standardized as different isolation methodologies can have negative effects on downstream PCR testing. Furthermore in order to eliminate variability with "home-brew" assays whenever feasible, the PCR genotyping methodologies used in the LabNet should be standardized to commercially available products.

Dr Sikkema recommended that reference sera should be derived from immunized subjects because of much higher titres than in naturally infected individuals and that such a reference should be of a volume so that it can be used globally in every assay for maximal assay standardization. He indicated that Merck was preparing a large pool of serum from recipients of Gardasil® and that a portion of this could be made available as a "standard". He also suggested that proficiency panel representing low, mid, and high antibody titres should be prepared to ensure that laboratory testing can match a range of antibody concentrations representative of current practice. He also recommended that consistency in detection method and readout are paramount for assay standardization.

The majority of the group considered that sera from naturally infected women should be used in the preparation of International Standards although all agreed that sera from vaccinees would be useful as working standards. Many other vaccine programs utilize reference standard sera from vaccinees (e.g. pneumococcal, meningococcal, pertussis), although many viral vaccine programs use sera from naturally occurring infection. This has proven useful for many programs where the natural infection elicits a very high titer immune response that is similar in magnitude to immunization. However, this is not the case for HPV, where immune responses to natural infection are lower than that observed for immunized subjects.

The pros and cons of commercial and in-house assays were discussed. Some of the participants considered that commercial assay kits are too expensive for many labs. Whereas others prefer to use commercial assays as QA and optimization has already been done by the manufacturer. It was therefore proposed that some assays should be selected with associated

high QC/QA demands. This in conjunction with standards and training would be key factors to satisfactory laboratory performance. Some considered that with appropriate controls and satisfactory performance in proficiency panels then an assay should be acceptable.

The recommendation of a limited number of assays for use in LabNet is problematic as the laboratories have a range of expertise and are already following different cohorts so cannot change methodology as comparable assays are required if patients are to be followed for 20 years.

It was suggested that WHO investigate with manufacturers whether assay kits could be selected from a range of manufacturers.

**Dr M. Deschamps** reviewed the HPV assays in use at GlaxoSmithKline (GSK), Belgium. Her presentation focused on antibody testing as DDL performs the HPV DNA testing for GSK (discussed by LJ van Doorn above). GSK uses the direct anti-HPV-16 and anti-HPV-18 ELISA as a primary immunogenicity endpoint and the HPV-16 and -18 pseudovirion-based neutralization assay (PBNA) as a secondary immunogenicity endpoint in GSK's clinical trials. Both assays are fully validated according to ICH guidelines, are stable over time and there is an excellent correlation between ELISA and PBNA. In the EIA, highly purified vaccinal L1 VLP are used as coating antigen and 8 two-fold serial dilutions starting at 1/100 are assayed. Serum titres are calculated against a reference serum standard using a 4-parameter regression. Titres around the cut-off are repeated for confirmation. The Limit of detection (LOD), Limit of quantification (LOQ) and cut-off are defined. Other parameters have been assessed including precision, linearity/recovery, specificity and positive predictive value. The Schiller pseudovirion neutralization assay is used and all parameters have been validated. Specificity was demonstrated by pre-incubation of an anti-HPV-18 neutralizing serum with HPV-16 and -18 VLPs. Only the HPV-18 VLPs are able to deplete the anti-HPV-18 response as measured by EIA or PBNA. Analogous results were obtained for anti-HPV-16 neutralizing serum. The EIA and neutralization results obtained in a clinical trial showed  $\geq 98\%$  of seropositivity for both HPV-16 and HPV-18 and comparable EIA and neutralizing antibody profiles for both HPV-16 and HPV-18 for up to 5.5 years. The correlation between the EIA and neutralizing antibody titres was further shown to be maintained for both HPV-16 and HPV-18 across all time points post vaccination and across all age ranges in all clinical studies (immunogenicity and efficacy). Finally a correlation between serum and cervical mucosa GSK ELISA antibody levels for both HPV-18 and HPV-16 was also observed.

Dr Deschamps suggested that any serology assay selected and used by the WHO HPV LabNet should target more than one epitope to ensure optimal correlation with the neutralization assay. Furthermore, if the assay uses vaccine antigen, it should correlate with an assay which does not use vaccine antigen such as the PBNA in order to avoid any bias which may occur through the use of antigen homologous to the immunogen. The WHO HPV LabNet-selected assay should be properly validated according to accepted guidelines (e.g. ICH guidelines). Dr Deschamps also recommended that the WHO International Standards for HPV antibody be prepared from naturally infected individuals. With respect to nucleic acid amplification (NAT) assays, Dr Deschamps recommended that HPV DNA tests be applicable to cervical swabs and biopsies, be validated according to guidelines, be broad spectrum and HPV type-specific. For both the HPV serology and NAT assays, she also recommended the utilization of validated reagents and materials and that the assays be performed by certified operators. Finally, the HPV serology and NAT assays should be constantly controlled through the use of controls on every plate, the use of acceptance criteria and regular assay of proficiency panels.

**Dr J. Shih** next described the development of prophylactic HPV Vaccines at Xiamen University and YST Biotech Inc in Xiamen, People's Republic of China. HPV L1 protein is expressed in *Escherichia coli*, purified from inclusion bodies and then renatured to give VLP. A bivalent vaccine containing HPV-16 and -18 VLP is going into clinical trials. The company is also developing a quadrivalent HPV vaccine containing types 6, 11, 16 and 18 as well as a hexavalent vaccine which also contain types 52 and 58. The HPV VLPs are well characterized including analysis by analytical ultracentrifugation and dynamic light scattering. A high throughput neutralization test using HPV-16, -18, -6, and -11 pseudovirions has also been developed. The antibody responses to HPV-16 and -18 VLP in monkeys have been investigated in neutralization and capture assays.

### **Session 3 Progress in development of International Standards and potential needs for standardization**

**Dr D.E. Wilkinson** (NIBSC, UK) reviewed the ongoing and proposed standardization projects for HPV testing being undertaken by NIBSC on behalf of WHO. One project which has been completed is the characterization and assessment of suitability of International Reference Reagent for HPV-16 antibody. Additional projects which have been proposed and which will aid the work of the WHO HPV LabNet are: 1) development and establishment of

an HPV-18 Antibody International Standard; 2) development of serum proficiency panels for HPV serology; 3) supply of monoclonal antibodies for use in the quality control of HPV VLP and 4) production and supply of high-quality VLPs and/or PsV for use in serological assays. These projects will be undertaken in collaboration with WHO HPV LabNet members and with research laboratories. The project to develop an anti-HPV 18 International Standard will be initiated later in 2008 and Dr Dillner has agreed to conduct donor screening and the collection of the serum required. During the studies to identify suitable donations for this standard, it is hoped that other donations suitable for inclusion in a serum proficiency panel will be identified. A panel of monoclonal antibodies (McAbs) suitable for the assessment of the quality of VLPs has been offered by Dr Christensen. These are: H16.V5- Positive control for HPV-16 VLPs; H18.J4- Positive control for HPV-18 VLPs and D9- Negative control (reacts with denatured VLPs).

High-quality VLPs and/or PsV are required for serology assays but most laboratories are unable to produce them. NIBSC currently does not have capacity to produce VLPs or PsV for universal use but can store and distribute these materials. Representatives from both GSK and Merck indicated that they were prepared to enter into discussion with WHO on the provision of VLPs for use by the WHO HPV LabNet. Candidate International Standards for HPV-16 and 18-DNA have been assessed in a collaborative study and the report will be submitted to ECBS for consideration in October 2008. Proposals for additional DNA standards for HPV types 31, 33, 45 and 58 are also being considered. The formulation and freeze-drying process have already been developed and we can learn from and improve upon the protocol and logistics of the current collaborative study. NIBSC is also preparing C33A DNA diluent for distribution with the HPV DNA International Standards as this has been identified as essential for their proper use.

The development of cell-based process controls is also being considered for distribution as NIBSC reagents. Although clinical samples are ideal for use as process controls, they are not practical. SiHa cells which contain 1-2 HPV-16 genomes per cell are an option. NIBSC has experience in the freeze-drying of whole cells for cell-based controls used in flow cytometry. Freeze-drying of cell-based process controls would facilitate their long term storage and shipment.

Points made during discussion include: 1) Individual WHO HPV LabNet members should identify and share sera more rapidly for use in interim proficiency panels. 2) Dr Dillner is to collect sera from naturally infected individuals independent of vaccines. 3) Vaccinee sera

could be established as a secondary standard. 4) Dr Galloway suggested that the LabNet focus on VLP-based EIA as this is the easiest technology to implement in most laboratories. The WHO HPV LabNet needs to identify a source of VLPs that everyone can use. 4) Availability of VLPs and monoclonal antibodies is essential.

**Dr Ferguson** then described the project to characterize and establish the International Reference Reagent (IRR) for antibodies to HPV type 16. The IRR is a freeze-dried preparation of pooled serum obtained from three women naturally infected with HPV type 16 only. The sera were collected by Dr Joakim Dillner with appropriate ethical permission and consent obtained. Eleven laboratories from nine countries participated in the WHO collaborative study to assess the material. Five laboratories performed neutralization assays based on HPV-16 PsV and all eleven participants performed immunoassays based on L1 VLPs derived and purified from a baculovirus expression system, yeast or *E. coli*. The candidate standard had titres which were low compared to the sera from vaccinees in neutralization tests. However, the low neutralization titre of the candidate standard is comparable (1:200) to other sera from naturally infected individuals. The data were analysed as parallel line assays following an appropriate transformation (replicate assays were analysed independently as assays). Laboratory mean potency estimates were calculated as geometric means. Overall mean potencies were calculated as the geometric means of the laboratory means. Inter-laboratory geometric coefficients of variation (% GCV) were also calculated. This material was considered suitable for use in immunoassays and in neutralization tests of adequate sensitivity. At the WHO ECBS meeting, 8-13 Oct 2007, the candidate standard was adopted as the first International Reference Reagent for anti-HPV 16 antibodies with an assigned potency of 5 units per ampoule or 10 units/ml when reconstituted as directed in 0.5 ml distilled water. Dr Ferguson then explained why this material was established as an International Reference Reagent rather than an International Standard. This was because it did not meet the stringent criteria for an International Standard to ensure long term stability as the candidate standard. The candidate standard had a higher residual moisture content, as determined by the Karl Fischer method, than observed generally in freeze-dried serum/plasma fills. Additional studies undertaken using non-invasive technology then demonstrated that the ampoules were not of equivalent residual moisture. The resulting concern is the potential impact on the long-term stability of this preparation. Although initial stability studies did not demonstrate differences in potency other than apparent difference between -70°C and -20°C, which would be expected to be very similar. Additional studies are

therefore being undertaken in which high and low moisture ampoules from stock stored at -20°C are being identified by non-destructive testing. Ampoules from each group will be assayed together for antibody content against ampoules stored at -70°. The additional stability studies will be completed and reported back to the ECBS for consideration of the establishment of this material as International Standard (IS). The unitage assigned at that time will be the same and it will then change from units (WHO units) to International Units. The IRR for HPV-16 antibodies may be used for the calibration of in-house or working standards. This could be in a single laboratory or in a group of laboratories. The number of independent assays is chosen so that the combined relative potency estimate has the desired precision. Dr Ferguson emphasized that it is preferable that replicates are made from independently repeated dilutions of freshly made solutions and not from sub-sampling from a single dilution series. All valid potency estimates for the candidate standard are then combined to produce a final mean potency estimate with confidence limits.

**Dr Wilkinson** next described the collaborative study which has been undertaken to establish WHO International Standards for HPV-16 and 18-DNA nucleic acid amplification technology (NAT) Assays. The candidate standards are full-length genomes cloned into pBR322 that were quantitated with high precision by Dr. Cosette Wheeler (University of New Mexico HSC, USA) and then supplied to the NIBSC specifically for the collaborative project. The candidate standards were subsequently formulated at NIBSC in 10mM Tris buffer pH7.4, 1mM EDTA and 5 mg/ml trehalose to contain  $1 \times 10^7$  genome equivalents (GEq)/ml of HPV 16 or HPV 18 in a human C33a cellular DNA background at a final approximate concentration of  $1 \times 10^6$  GEq/ml. Each candidate standard was dispensed in 0.5ml aliquots into 3ml DIN ampoules and freeze-dried. The bulk materials from which each standard was prepared were included in the study along with trial fills of HPV-16 and -18 DNA which produced sub-optimal freeze-dried plugs. C33A DNA diluent containing  $\sim 1 \times 10^6$  genomes/ml in dH<sub>2</sub>O was supplied to all participants for use in the preparation of dilution series to provide a constant cellular DNA background. Participants were requested to perform four independent assays using a fresh tube/ampoule of study sample in each assay. Generally, individual laboratory mean estimates are grouped around the theoretical  $1 \times 10^7$  HPV GEq/ml. The spread of results ( $\pm 2 \log_{10}$ ) is not unexpected for this type of study in which a variety of qualitative and quantitative assays are used. The agreement between laboratories is markedly improved when the activity of the study materials are expressed relative to the candidate standard. The overall mean  $\log_{10}$  relative potencies (in which the

bulk preparations and trial freeze-dried samples are expressed relative to the candidate International Standards which were assigned a hypothetical unitage of  $7.0 \log_{10}$  units/ml) show a slight drop of potency on freeze-drying of  $0.15 \log_{10}$  for the HPV16-DNA candidate and  $0.02 \log_{10}$  for the HPV18-DNA candidate. These are relatively small losses for measurements where assay precision is likely to be no better than an order of  $0.3 \log_{10}$ . A unitage for assignment to these materials has to be proposed for these materials and Dr Wilkinson raised the issue of whether this should be GEq/ml or International Units (IU)/ml. She informed participants that WHO International Standards are generally assigned arbitrary unitages in International Units and that should genome equivalents be proposed this would necessitate the use of a single reference assay which should not be a biological assay and should be derived from, and traceable to, physicochemical procedures. Following some discussion, it was proposed that a unitage in International Units be proposed but that the calculation of GEq from the DNA content be included in the “Instructions for Use” so that traceability was available. Dr Wheeler confirmed that the sequences of the plasmids supplied for the development of the standards are the same as that of DFKZ and that there are no known errors at this level. Dr de Villiers suggested that the “Instructions for Use” include detailed instruction concerning the precautions which should be taken during opening the ampoules to avoid contamination of the area with plasmid DNA.

Additional points made during discussion include: 1) The HPV LabNet should consider developing standards for HPV types 6 and 11. For uncommon HPV types, it may be difficult to find suitable sera from naturally infected individuals and the use of vaccinee sera should be considered as an alternative, provided that possible bias of results obtained with the selected serology assay can be excluded. 2) Development of DNA standards for additional single HPV types is needed. 3) In order to mimic a cellular DNA background during NAT assays, it is necessary that C33A DNA diluent is supplied alongside each HPV DNA International Standard.

#### **Session 4 Building the capacity of the global HPV LabNet**

**Dr Dillner** gave his views on harmonizing global HPV laboratory practice from the perspective of a Global HPV Reference Laboratory. He considered that the lack of standardized methods is an obstacle in the progress of HPV-related cancer eradication. Standardization of laboratory procedures and methods has been accomplished for a number of other vaccine-preventable diseases by launching WHO laboratory networks. The goals of the HPV LabNet that have been defined by the WHO are to facilitate the implementation of

validated, standardized laboratory procedures, which requires quality assurance and proficiency testing; training personnel and supplying equipment if required; and providing a network for surveillance. The HPV LabNet should also promote a widely recognized understanding of the possibilities and limitations of HPV laboratory assays in assisting vaccine development, vaccination implementation and surveillance of HPV. The need for HPV laboratory assays will differ between countries and the demands on the assays may also change with time. Stringent design of population sampling strategies, that will allow international comparison are important. Other issues are choice of sample type, sample handling, testing, data analysis and reporting. Every step requires quality assurance (QA). Thus, QA applies not only to the assays itself, but to the entire operation of the laboratory. Quality indicators (QI) measure quality in a quantifiable and reproducible manner. The QIs and how they should be measured must be defined and be the same globally. Important QIs will include the use of standards and assays of proficiency panels. Laboratories will need to measure quality indicators and see their improvements over time. When QIs are measured in the same manner, there is international bench marking where QI measurements are compared with others and experience can be shared so that laboratories gain improvements without everyone having to experience the same quality problems.

Standardizing Standard Operating Procedures (SOPs) may not be a priority at this time as this is time-consuming and may stop development work or the rapid improvements currently being undertaken by many investigators around the world. It is emphasized that common standards and quality indicator measurements are independent of the specific assay used and will thus promote progress in assay development by enabling the sharing and comparing of results and experiences.

It will be important that standards with assigned unitages in IU are used. Every laboratory will not be able to calibrate working standards so this will have to be facilitated by the global laboratories and NIBSC. Good progress has already been made with the development of ISs, proficiency panels and the drafting of the laboratory manual. The global laboratories should establish best practices or at least find examples of systems that work. The Global Reference Laboratory in Sweden has responded to the instructions from the WHO that the HPV LabNet should provide a network for surveillance, and is piloting HPV surveillance systems in Sweden using both HPV antibodies and HPV DNA detection methods applied to organized surveillance systems. In the future, guidelines for design of HPV surveillance may be written and proficiency criteria for HPV DNA testing will be determined. As discussed earlier in the

meeting, other tasks include: the development of proficiency panels for serology and implementation of a serology proficiency study, determining an internationally standardized "cut-off" value; ensuring a standard supply of high quality VLPs for serology and high quality pseudovirions for neutralization along with positive and negative control monoclonal antibodies for serology and VLP quality control. Dr Dillner also emphasized the need for a standardized reporting system for providing data to WHO.

Dr Dillner also highlighted the need to ensure that the world is aware of the WHO efforts in this field to avoid duplication. Mechanisms to achieve this include the laboratory manual, the newsletter and the WHO HPV website. He also indicated that it would be helpful to have a LabNet member from South America, but otherwise agreed that it is necessary to ensure that the current LabNet is functioning well at the Regional Laboratory level before it is expanded to include laboratories at the National level.

It was emphasized that all assays, including newly developed techniques or imported assays from other laboratories, should be validated.

An aim of the network is to inform public health authorities in different countries about the possibilities of laboratory-based HPV surveillance and the need for international comparability. This requires interactions with local authorities and the LabNet should be able to train the national labs who are not in the HPV network.

It was suggested during discussion that criteria need to be set up for responding to discrepancies between results of labs. The need to evaluate assays and then to give advice was emphasized. It was suggested that assay kits could be assessed in global studies for subsequent recommendation to national labs.

**Professor A-L Williamson** (Regional WHO HPV RL, University of Cape Town, South Africa) gave her perspectives from a regional HPV Reference Laboratory in a developing country setting and reported that her laboratory is relatively well resourced which is not typical of an African laboratory. Developing countries have a major burden of HPV-related disease but cervical screening programs are under-resourced or non-existent in most of Sub Saharan Africa. Treatment is also under-resourced and women are dying from a preventable and treatable disease. There is a high burden of disease in Africa with incidence rates of 32-87 cases of cervical cancer per 100,000 women. The South African cervical cancer control policy is to provide three free screenings of cervical smears within a woman's lifetime with 70% coverage by 2014. This policy would reduce disease by 50 % if implemented properly

but unfortunately this is not the case. HIV increases the risk of cervical cancer. HIV-positive women in Johannesburg present with invasive cervical cancer almost 10 years earlier than HIV seronegative women. HIV positive women are at a greater risk of lower genital tract neoplasias including vulvar and anal cancers. It is estimated that there are 5.41 million South Africans infected with HIV and 30% of ante-natal women are HIV-positive. Whereas now women are dying of HIV before they develop cervical cancer, it is likely that with the introduction of anti-retroviral drugs, women will live longer and will not die before they develop cervical cancer. There are large gaps in data for HPV vaccine implementation. Gardasil® is registered in 15 African countries and Merck also is seeking WHO prequalification for Gardasil®. HPV Vaccine Trials in Africa include an assessment of the prophylactic efficacy of Gardasil® against external genital lesions in men and a phase II study of safety, tolerability and immunogenicity of Gardasil® in HIV-positive women (with stratification by viral load and CD4 count). Knowledge gaps are: 1) Data on HPV vaccine efficacy in men and women who subsequently become HIV positive. 2) More data is needed on HPV types associated with cervical cancer in Africa. 3) Burden of genital wart disease particularly in HIV positive people. 4) Cost analysis of impact on the introduction of HPV vaccines into the public sector. 5) Feasibility of introduction of HPV vaccine targeted at 10-12 year old girls. In summary, Africa needs HPV vaccines and workshops to inform public authorities.

**Dr Dillner** presented a report of a WHO proficiency study for HPV DNA testing. In principle, accrediting bodies mandate that clinical laboratories participate in annual proficiency panel studies to maintain accreditation. Proficiency panels are manufactured and studies are organized by a number of different organizations and companies. It must be remembered that proficiency panels test only ONE step in the analysis chain of surveillance studies. Sample handling; such as collection, transportation, pre-treatment, safety measures against mix-up and data handling, is also important. The 1<sup>st</sup> HPV DNA proficiency panel was formulated in Dr. Dillner's laboratory and the considerations for its composition included: 1) Which of the 14 oncogenic and 2 benign HPV types to include? Fourteen oncogenic and two benign types were chosen. 2) What are the requirements for sensitivity in detecting HPV DNA types in mixed infections/detection of multiple infections? 3) There should be high capacity to detect wrong typing (e.g. through cross-hybridization, etc.). 4) The panels should work for all HPV DNA detection and genotyping systems known today.

Dr Dillner suggested some Quality Indicators based on HPV DNA proficiency panel testing. Sensitivity and specificity of HPV DNA testing should be evaluated at least annually using a blinded proficiency panel issued by the HPV LabNet. Results should at least conform to what is considered useful for the intended purpose, which is HPV surveillance.

Possible Quality Indicators could be that a laboratory deemed proficient in HPV DNA testing should not have mis-typings or false positives. Furthermore, laboratories should demonstrate an analytical sensitivity of 50 genome equivalents for HPV-16 and HPV-18 and of 500 genome equivalents for the other HPV types. Seven HPV LabNet members plus two WHO collaborating centres, one of which was NIBSC, participated in the first study to characterize the WHO HPV DNA proficiency panel. As the assay methods employed in the testing were known, this study also served as an evaluation of the assays used. Most laboratories had adequate results but a few laboratories may wish to consider changing method. Some laboratories used in-house assays and some used commercial assays. The Roche Linear Array HPV Genotyping Test as well as the PGMY and GP5+/6+ platforms were shown to have a low sensitivity for detecting HPV-39 and HPV-68. There were also occasional false positives reported in the study. It is proposed that the proficiency study be repeated using an extended HPV DNA panel and that not only WHO HPV LabNet laboratories participate, but that external laboratories be invited to participate via advertisement on the WHO website.

During the discussion, it was suggested that if an HPV type was included in a mixture of HPV types, it should also be provided individually. The issue of the clinical relevance of the levels of detection required was raised by Dr Wheeler, who indicated that there is no clinically irrelevant amount of virus in relation to vaccines as any infection by HPV-16 or HPV18 is currently considered a vaccine failure. Dr Unger suggested that a virus level in a proficiency panel consisting of plasmids is considerably easier to detect than the same virus amount in clinical samples. Nevertheless, it is accepted generally that proficiency testing raises the quality of testing. It was suggested that data on assay kit performance could be generated through their inclusion in the next proficiency panel studies and perhaps companies should be asked to donate kits. Where laboratories are failing to detect some types, this could result in some types being systematically underestimated in surveys. It is acknowledged that the use of plasmids in proficiency studies only assesses the detection step in the assay. Some participants suggested pooled clinical samples should be included to control for extraction although different samples would have to be included in every study making it difficult to compare results over time.

**Dr TQ Zhou** outlined the proposed WHO HPV LabNet workplan for 2008-2009. This is essentially a continuation of the work initiated in the first year of the WHO HPV LabNet in developing standards and harmonizing assays for HPV antibody and DNA testing. The WHO HPV LabNet is expected to evaluate and implement reliable assays which should be generally applicable to all laboratories in different settings. The WHO HPV LabNet will also implement the established WHO International Standards and Reference Reagents. A second proficiency study for HPV DNA testing will be undertaken which will include external laboratories who are interested in participating. Panels for HPV serology will be developed with a proficiency study on HPV antibody testing to follow. Confirmatory testing is an important aspect for Quality Assurance and Control and the HPV Regional Reference Laboratories are expected to submit samples for confirmatory testing in the two HPV Global Reference Laboratories. The main task for 2008-2009, however, will be to reinforce capacity and competency for HPV testing in WHO HPV LabNet laboratories in order to provide services for HPV surveillance and monitoring of vaccination impact. Capacity-building activities include ensuring that Regional Reference Laboratories are proficient in both HPV serology and DNA detection and genotyping. Implementation of a QA system in each Reference Laboratory is another high priority of the WHO HPV LabNet. This will involve training and guidance to LabNet members to ensure that assays are appropriately controlled. It is envisaged that the draft of the Global HPV Laboratory Manual will facilitate this by providing guidance in HPV testing. The Global HPV Laboratory Manual, which when adopted will be available to HPV laboratories worldwide, will be particularly important in training prospective national laboratories in developing countries that are involved in HPV surveillance and vaccination monitoring. The final version of the laboratory manual is expected to be available by the end of 2008 with possible approval in 2009 for publication as a WHO IVB document. This will be a living document which will be revised and updated as advances in the field are made.

Training will be prioritized to ensure that the Regional Reference Laboratories are fully equipped and proficient in performing the necessary testing within the WHO HPV LabNet. The more experienced WHO HPV LabNet members may have a role here by providing training to those laboratories in which gaps have been identified in the proficiency study or who are currently not set up to conduct both HPV DNA and serology testing. This exercise could be used as a template for developing training materials and curricula for future WHO HPV LabNet training programs. The LabNet will also build up the synergy network through

communications via the LabNet Newsletter and the WHO HPV LabNet website. It is hoped that the WHO HPV LabNet SharePoint will become a useful tool and used by the members. Additional LabNet Regional Reference Laboratories are needed, e.g. South America, and applications have been received and the election process is ongoing.

In summary, the WHO HPV LabNet will continue to build, strengthen and improve the capacity of the LabNet in HPV testing in order to provide a network of qualified laboratory services to meet the expected demands in HPV surveillance and vaccination monitoring, especially in low and middle income countries where capacity is currently limited. This will be achieved by: implementing validated and standardized laboratory procedures; developing a Quality Assurance system in each laboratory; participating in proficiency testing for HPV serology and DNA detection and genotyping; and training personnel in HPV laboratory practices.

It was suggested during discussion that the LabNet Terms of References (TORs) should be continued with some individual projects as in the previous year until all laboratories obtain equivalent expertise through capacity building.

The site for training was discussed as to whether it should take place in a laboratory with established infrastructure and assays or in the laboratory of the trainee where all the problems and deficiencies could be seen. The number of trainees accommodated at any time was also discussed as previous experience has demonstrated that trainees must learn at the bench. It was agreed that both approaches were needed with initial training in a training centre followed by a visit to the regional labs to identify gaps. Training capacity then needs to be increased in anticipation of the need to train National Reference Laboratories. To this end, Regional Reference Laboratories need to be trained as trainers and how to evaluate the training. This could be accomplished in collaboration with the Global Training Network already established by WHO.

Dr Wheeler suggested that there was a need to define what surveillance will take place. It was proposed that this is likely to be sentinel surveys. Once such sites are identified, it will be clear where the capacity needs to be built. It was also queried how will serology assist in surveillance and suggested that further study in this subject should be conducted in order to gather more data on the value of serology, as vaccine manufacturers will look at waning antibodies over the next 20 years.

Dr Unger suggested that there is a need for serology and DNA testing in certain settings but we do not know how vaccine and studies will roll out. For now we need to get manuals together and get proficiency studies sorted out.

The WHO Regions would like to establish workshops to standardize methodologies in Regional Reference Laboratories and then have National Reference Laboratories trained at these workshops or on site. This will be more thoroughly considered when WHO HPV LabNet-standardized assay methodologies and consistent training materials and curricula are available.

Dr Irwin indicated that any recommendations on vaccination may not be accompanied by recommendations on surveillance. As suggested by the last HEAG meeting in September 2007, a WHO consultation is required to discuss guidelines and recommendations for HPV surveillance and vaccination impact monitoring.

## **Session 5 Role and function of WHO HPV LabNet in supporting global HPV vaccine introduction**

**Dr E Unger** outlined the role of WHO HPV LabNet in HPV surveillance before and after vaccine introduction and indicated that HPV surveillance goals must be established first. The LabNet is only part of the process and its activities need to be integrated with public health authorities, industry, nongovernmental organizations and academic partners working together to achieve the goals set out by WHO. She noted that it must be recognized that the WHO HPV LabNet will be different from other WHO-established laboratory networks as its disease target is primarily cervical cancer not HPV infection. In other words, The WHO HPV LabNet is looking at rare consequences of persistent HPV infection and not simply at infection. The health problem is therefore chronic; with no threat from acute outbreaks which other WHO laboratory networks normally address. In addition, the costs of HPV vaccination and testing are much higher than for other agents targeted by vaccination and high-volume testing is required to evaluate HPV in populations.

Possible goals of surveillance are to determine type- and age-specific HPV prevalence in populations, and cervical cancer incidence, age-specific incidence and HPV type distribution. This data may be required to demonstrate to national decision-makers that the HPV vaccine matches their needs; to direct the age of vaccination; and to provide baseline data to benchmark the impact of vaccination. While type-specific DNA testing methods would provide the most useful information, in some settings HPV seroprevalence may be sufficient

to guide vaccine implementation. A dual approach to cervical cancer control, involving both screening and vaccination, appears likely and it may not be necessary to distinguish the contribution of vaccination versus screening. Therefore it may be reasonable for the WHO HPV LabNet to provide expertise on sampling and testing, to build capacity for HPV testing in regional and local laboratories and to train local laboratories to integrate high-risk HPV detection and cervical cancer screening before vaccination programs are introduced.

Possible goals of surveillance after vaccine introduction are to monitor vaccine efficacy and vaccine uptake. Serology could be a marker for vaccine uptake. The intermediate end-points for post-vaccination surveillance are not cost-effective and not likely to be adopted in low resource settings. These include type-specific prevalence in the population, incidence of cervical intraepithelial neoplasia (CIN) 2/3 and type-distribution in CIN 2/3. Monitoring CIN 2/3 is particularly challenging because of imprecision of the endpoint. Changes in screening and intervention after vaccine initiatives could paradoxically increase detection despite an actual decrease in cases. There is recognized inter-observer variability in CIN 2/3 diagnosis, and the population denominator, which is required for incidence determinations, is difficult to ascertain in the absence of systematic screening.

Dr Unger considered what factors prevent HPV testing from being widely available and suggested that the lack of trained personnel, laboratory facilities with required infrastructure, affordable testing reagents such as VLPs and standardized typing reagents, standards and proficiency testing, all contribute. Laboratory initiatives can improve surveillance through developing and implementing 'faster, cheaper, better' tests. The LabNet can assist in validating novel non-invasive sampling methods to monitor vaccine uptake (e.g. antibodies in saliva), standardize and validate self-sampling methods for HPV DNA testing such as dry swab or paper smear, improve high-throughput methods for extraction and type-specific detection, and use proficiency testing to determine type-specific reproducibility of all assays.

Dr Irwin indicated that surveillance activities collating cytologic abnormalities have already been initiated by IARC and ICO. Country-specific information is available on the HPV website. There is, however, limited data from EMRO and she questioned whether this was due to a lack of pathology and cytology laboratories. Not every country will require the same level of evidence, but in countries that lack sufficient data to inform decision-makers there is a role for the WHO HPV LabNet to assist in surveillance. Studies in most countries of the world indicate that about 70% of cervical cancer is due to HPV-16 and -18 and both vaccines provide high efficacy against persistent infection and precancerous lesions due to these types.

Countries lacking such data but that may be concerned about different HPV type distribution in their cancer cases, may wish to assess HPV types associated with cervical cancer in population based samples; if this approach is affordable and feasible and if such information would guide HPV vaccine introduction decisions. For countries with no laboratory facilities, regional laboratories could perhaps help establish basic studies to get some data and then, in the future, build national capacity. It is acknowledged, however, that in some countries sample collection is an issue as it is difficult to get non-married women to go to clinics. She also suggested that differences in regional prevalence may in fact be due to differences in testing methodology, so QA of assays needs to be verified to determine reliability of prevalence determinations.

Dr Williamson said that in her experience it is possible to enlist clinicians to assist in specimen collection to estimate population prevalence in countries with no data.

Dr Ennaifer-Jerbi (Regional WHO HPV RL, Tunis Pasteur's Institute, Tunisia) reported that all EMRO countries have cytology and histopathology laboratories, however they do not consider HPV genotyping to be necessary to decide whether or not to implement HPV vaccination. The decision to vaccinate should be based on a high incidence of cervical cancer. When the burden of cervical cancer is high, HPV detection and typing may be a waste of money. In some instances, treatment of cancers may be less than the cost of vaccine. Dr Wheeler agreed that vaccine implementation requires primarily determination of the burden of disease. Screening is not where the LabNet needs to go. She proposed the need for standardized reagents and generic kits to support defined surveillance, and also saw difficulties in studies to demonstrate type-replacement following vaccination for HPV 16 and 18 as such studies will be testing 100,000 individuals.

Dr Irwin emphasized that as an alternative to serologic monitoring, vaccine coverage can also be determined from a vaccine registry.

Dr Kasolo from WHO Regional Office for Africa (AFRO) suggested that the introduction of HIB vaccines may be the closest parallel to the HPV situation. Most countries refused to implement HIB vaccination until they had baseline data on disease in their countries.

However, there are now 21 sentinel sites and the decision to introduce vaccination by some countries in a region has helped other countries to accept the vaccine. There was a similar situation for rotavirus vaccine. So some HPV testing is justified, but he then queried whether extensive complex testing was required. Dr Villa agreed that indeed in several developing

countries governments are proposing to gather information about HPV types in the region. For instance, HPV 58, which is not contained in the present prophylactic vaccines, is a prevalent type in South America and Asia . However, although the HPV type-specific prevalence may vary considerably in asymptomatic or low-grade cervical smears, in cancers HPV-16 and -18 are the most prevalent HPV types worldwide. It is important to convey these messages to avoid delay of vaccine implementation in countries with the highest rates of cervical cancer.

Dr Sahli (Regional WHO HPV RL, CHUV, Switzerland) indicated that the LabNet should not engage in HPV screening and clinical diagnosis, but should focus on epidemiological studies and surveillance, as demands differ for these different applications of HPV testing.

Dr Garland suggested that for certain types of low-risk HPV infection, such as HPV-6 and -11, cases of warts should be counted.

During discussion, it was indicated that WHO cannot select a commercial assay from a single company at the current stage but evaluation of available kits could be undertaken through proficiency studies in expert laboratories. Dr. Galloway proposed that standardized materials and assays be made available to laboratories including sources of VLPs or primers.

There is lack of faith of current surveillance data for HPV, as data might be generated by using assay methodologies with different performance. The use of standardized assay methods are required to ensure consistent and qualified data.

**Each of the WHO Regions then presented the perspectives and needs from the regions in supporting HPV vaccine introduction.**

**Dr AM Bispo de Filippis** gave the view from Regional Office for the Americas (AMRO)/ Pan American Health Organization (PAHO). Cervical cancer persists as a significant public health problem in Latin America (LA) and the Caribbean, despite the long standing availability and application of Pap smear screening in most member states. Various publications indicate that 70% of cases are caused by HPV-16 and -18. The ten most frequent HPV types in women vary within the region. An HPV surveillance system is not in place in LA. There are sentinel sites for surveillance in the USA and some HPV projects are taking place in Canada. Research studies are underway in 8 countries and 13 countries have state or private HPV DNA Laboratory capacity. However, sentinel surveillance strategies for HPV among women are needed in some countries of the Americas Region. There are plans within the Americas to: 1) estimate the incidence and prevalence of HPV types by geographic

location in both pre- and post-vaccination eras; 2) be supported by the WHO HPV LabNet with standardized procedures and Quality Control guidance; 3) gather baseline data for assessing the pre-vaccine HPV prevalence and compiling evidence for vaccine introduction and informing decision-makers on cancer prevention activities; 4) monitor the impact and effectiveness of HPV vaccine introduction; 5) provide evidence to determine whether or not policy changes may be indicated.

Within the Region of the Americas, there are 10 laboratories that are part of the polio network at specialized, national, regional; sub-regional and sub-national levels. There are 148 laboratories contributing to the measles and rubella network, 124 at the sub-national level, 21 at the national level, 2 regional laboratories and 1 global specialized laboratory. There are similar setups of PAHO laboratory networks for rotavirus and pneumococcal vaccine introduction. So there is a lot of laboratory expertise and infrastructure in the region. For HPV, the Centers for Disease Control (CDC), Atlanta, has been appointed as a Global Reference Laboratory and it is anticipated that two PAHO regional laboratories will be nominated along with 6 to 8 national laboratories. PAHO sees a need for the standardization of HPV testing methodology as most laboratories are using in-house assays. In support of immunization programs, PAHO facilitates the activities of the laboratory networks by: assuring data quality; assisting laboratories in accreditation; supporting meetings, training and courses; promoting research and test validation; and identifying and preparing funding proposals to mobilize financial resources in support of national and regional plans. The perceived challenges of the WHO HPV LabNet in the Americas region include a lack of standardized testing algorithm and routine testing and the high cost of reagents and lack of resources in supporting HPV surveillance and vaccine implementation. HPV is not a reportable infection, therefore public health information is lacking for HPV. Furthermore, there is a lack of public awareness of the association between HPV infection and cervical cancer.

It was agreed that laboratories already in the polio network are most likely to provide a foundation for the HPV network as they have experience with sequencing and molecular methods, even if different equipment is required. However, many academic HPV laboratories may not want to take on long-term surveillance activities.

**Dr F Kasolo** then presented an overview of HPV laboratory activities in the African Region (AFR). Cervical cancer is responsible for a significant burden of malignancy in Africa. A number of studies have been conducted on cervical cancer and HPV although no formal

surveillance is in place. A number of countries have established cancer registries as a means of quantifying the burden of cancers. A Regional HPV Reference Laboratory from South Africa has been designated with an additional four laboratories from Senegal, Uganda, Kenya and South Africa expressing interest in joining the global WHO HPV LabNet. These laboratories have in place equipment and infrastructure to support HPV work. It is envisaged that these laboratories will need orientation in the various HPV-related technologies before joining the WHO HPV LabNet. HPV vaccination is a long way off as neither vaccine is licensed in the majority of the African countries. There is limited interest in investing in an AFR HPV LabNet as countries and institution do not see the need to do so at the moment. The way forward in strengthening the WHO HPV LabNet in the region would be to develop HPV surveillance in a few countries, which will in turn act as models in the African Region. Also, there is a need to strengthen links between emerging HPV National Reference Laboratories and the Regional Reference Laboratory in South Africa in order to ensure quality HPV testing within the African Region.

During discussions it was stated that the HPV and cervical cancer program will be incorporated in the women's health divisions in order to increase its profile.

**Dr HJ Ahmed** next gave the EMRO (Regional Office for the Eastern Mediterranean) view on HPV vaccine introduction. The Eastern Mediterranean Region (EMR) is comprised of 22 countries with wide differences in socio-economic standing. The Gulf states are high-income countries but then there are middle- and low-income countries such as Somalia, Pakistan and Afghanistan. EPI programs are supported by GAVI in 6 countries and during 1995-2006 the coverage of EPI vaccination was around 80%. All 22 countries have laboratories which are part of the measles and rubella network including 2 Regional and 2 Sub-Regional laboratories. Each of the member laboratories is set up to do serology, 12 are able to perform virus isolation and 2 are set up for PCR assays. The measles and rubella laboratory network is functioning at a high level of proficiency. A systematic program of validation of samples is operating in the region and an accreditation program has been implemented. The Eastern Mediterranean Region measles laboratories also conduct polio work. The EMR also has a sentinel vaccine-preventable disease surveillance network in number of countries to provide evidence on disease burden and strain types for new vaccines such as rotavirus and bacterial meningitis. Information on cervical cancer incidence and mortality is limited however. WHO estimate the incidence rate of cervical cancer in the EMR ranges between 8 to 29.9 per 100,000 except in Djibouti, Somalia and Sudan where it is estimated to be > 45 per 100,000.

A few countries have done some research on HPV screening in academic institutions, however different approaches were used so prevalence cannot be compared between these studies. No country in the Region has introduced routine HPV vaccination and only two countries have so far licensed Gardasil® or Cervarix®.

EMRO activities towards HPV Vaccine introduction include: 1) organizing an HPV meeting in March 2008 involving at least 13 countries from the region; 2) building national interests by providing information on cervical cancer burden, the HPV types causing cervical disease and HPV vaccine availability; 3) creating multidisciplinary country teams that collaborate to provide information to policy-makers on HPV vaccine introduction; 4) assessing country programs on reproductive health and cancer screening and provision of health care services; 5) setting country selection criteria parameters followed by selection of candidate countries that can implement comprehensive HPV programmes. 6) establishing a sentinel surveillance network that can provide evidence on HPV burden for decision making process. 7) organizing regional and country level workshops to increase awareness and strengthen needed capacity. 8) drafting a regional strategy as well as country work-plans addressing disease burden and comprehensive prevention strategies including HPV vaccine introduction.

The Pasteur Institute in Tunis was identified in 2006 as the Regional Reference Laboratory. Later this year EMRO plans to conduct laboratory training workshop for the detection of HPV for 4-5 countries in order to establish a Regional HPV Laboratory Network.

As it would be advantageous to use SOPs and validated HPV assays in all laboratories, neither of which is currently available, the region does not know which assays to train on. EMRO needs to raise the interest of collaborators and donors for cervical cancer control programmes so that they can monitor the impact of HPV vaccine when introduced. The planned activities require seed funds but at present EMRO has no funds allocated for HPV activities so such activities may not be realized. Implementation of vaccination programs will be at the country level but for implementation to take place they need to have interest stimulated and information given

**Dr Y Jee** presented an overview of HPV laboratory activities in Western Pacific Region (WPR). WPR covers 37 countries and there are two HPV regional reference laboratories, one in Japan and one in Australia. The region also has 21 polio laboratories which can be built upon when it comes to expanding the HPV network. The geographical distribution of age-standardized cervical cancer incidence rate per 100,000 women varies depending on the

geographical area within Asia and in the South Pacific Region. A study to investigate the burden of cervical cancer and cost benefit analysis of HPV vaccination in Tonga has been undertaken and PATH has undertaken a demonstration research project in Vietnam. A project to genotype HPV isolates using DNA chip technology has been conducted in Korea. Four hundred seventy one samples were tested and this appears to be a sensitive diagnostic tool for the detection of HPV in cervical specimens. Another project has been undertaken in Fiji to establish the burden of cervical cancer and cervical intraepithelial neoplasia in women.

Gardasil® and Cervarix® are both licensed in 9 WPR countries. Some countries have already introduced vaccine into their immunization programs. Free vaccine is available in Australia for females aged 12-26, the age range for which the vaccine is licensed, but the program does not include funding for males. In New Zealand, there is a higher incidence of cervical cancer among Maori women but there is limited data on which HPV Types predominate in cervical cancer. Gardasil® is licensed in New Zealand and HPV vaccine will be included in New Zealand immunization schedules from 2008. Challenges which exist for the LabNet are the designation of National Reference Laboratories and the standardization of HPV laboratory methods.

The performance of laboratories through the development of quality indicators is essential as is a mandatory reporting system. The goal for each WHO Region should be to assess HPV laboratory capacity and the roles of the HPV LabNet in each region should be defined. The training and education of National Reference Laboratories is required.

During discussion it was highlighted that there is an urgent need to feed the network activities into regional plans now, before new biannual plan are implemented as otherwise it will not go in until 2010.

It was also emphasized that regions must strengthen activities in HPV and they must contact countries to make them aware of the issues. This could take place at regional meetings which should include all the focal points for immunization, STI, cancer screening. Doctors who could supply specimens should also be informed.

It was recommended that the report of this meeting and recommendations be sent to regional focal points and also the WHO Representative (WR) in each country.

Dr Dillner highlighted that the WHO HPV LabNet should identify important collaborators such as national agencies and others who will instigate surveillance programs, as the mission of the HPV LabNet to provide a network for surveillance can only be accomplished in

collaboration with the responsible agencies. As many of the regional laboratories are research laboratories not previously involved in public health work, they may require guidance on finding the appropriate contacts and setting up the collaborations.

Trainings on HPV testing, should be provided with priority given to the Regional Reference Laboratories in the anticipation that Regional Reference Laboratories will in turn train national and local laboratories in due course.

## **Session 6 Review of the Global HPV Laboratory Manual**

**Dr Dillner** presented a progress report for the development of the Global HPV Laboratory Manual. The outline of the manual was proposed in the 2005 WHO meeting report (4) and the preparation of the Global HPV Laboratory Manual is cited in the TOR for 2006 for the Global Reference Laboratory in Sweden. Dr Dillner examined other WHO laboratory manuals as potential models for the HPV laboratory manual. The WHO Measles laboratory manual has clear aims and relevant information presented for different users and the WHO Polio laboratory manual is highly informative with detailed SOPs including environmental conditions and handling of waste. It also includes relevant aspects of QA and laboratory organization. The responsibilities of laboratories at all levels of these laboratory networks are detailed along with criteria for laboratory accreditation. It was emphasized that these are mature laboratory networks and that the manuals are living documents. For example, the polio manual is currently in its 5<sup>th</sup> edition. The first edition of the HPV manual should therefore have a more limited ambition. The WHO polio LabNet supplies reagents for ELISAs as well as probes for hybridization. The Polio manual is therefore being used as a model document in the preparation of the HPV Laboratory manual. The assignment of chapters of the HPV manual had been discussed and agreed at the WHO HPV LabNet meeting in Lausanne in June 2007.

The group discussed various aspects of the issues to be covered in the manual and it was agreed that there should be a chapter on assay validation which would also detail the extent of studies required to implement an already established assay, as this will not be as extensive as that undertaken in originating labs.

Which assays to be included was discussed extensively as inclusion of a specific laboratory method may appear as endorsement of a method by WHO as a 'standard' method. It was agreed that the first draft of the Global HPV Laboratory Manual will explain how techniques work as well as quality assurance and validation issues. SOPs for specific methods may at

this time go onto the LabNet SharePoint as reference documents for the purpose of information/knowledge-sharing. It was also suggested that when there ultimately is selection of assays to be recommended by the LabNet, this should take into account the ease of implementation and overall cost to the local and regional laboratories. Dr Sikkema expressed reservations on the draft HPV Laboratory Manual, because of the level of specific information included and possible wide reaching implications in its use. For example, the manual may be seen to contain WHO-recommended criteria by official bodies such as the FDA. It was agreed by participants that the HPV laboratory manual needs to be rewritten and should at this time focus on more general issues.

The requirements for WHO accreditation and WHO LabNet membership was also discussed. WHO needs to establish a formal written accreditation process. This may include on-site visits and measurable quality indicator-based criteria that are analogous to those required by laboratories in the polio network e.g. processing a minimum number of samples throughout the year so that competence is maintained. Documentation ensuring traceability of samples from receipt to reporting, including calculations is also required. Adequate competency in proficiency studies is required and unacceptable results should mean failing inspection. Once the WHO HPV LabNet is mature enough, quality indicators should be developed to indicate the satisfactory performance of LabNet members. The availability and supply of critical reagents was also discussed. It was suggested that WHO should make a supply of critical reagents available while laboratories will buy generic reagents. It was noted that Intellectual Property issues would then need to be addressed. Once the logistics such as determining quantities required were considered, WHO needs to explore funds for supplying reagents to the LabNet. Proficiency panels along with availability of standards were also identified as crucial.

### **Closed session:        Recommendations**

Upon the conclusion of the meeting in a closed session, recommendations were identified to assist the WHO HPV LabNet in fulfilling its mission. These include:

#### **Recommendations for the WHO HPV LabNet**

1.        The proposed WHO HPV LabNet workplan for the next year was agreed, in general, by the members. In order to be prepared for future demands, the current priority of the WHO HPV LabNet should be given to capacity-building at the Regional Laboratory level.

2. The generic TORs defined in 2006 for the WHO HPV LabNet need to be revised in order to bring it in line with the current situation of the LabNet in HPV testing and monitoring of vaccine impact.
3. Communication between the WHO HPV LabNet members is to be maintained through at least annual meetings.
4. The LabNet newsletter should be published at 6 monthly intervals with contributions from all network members at regular intervals. The WHO HPV LabNet newsletter will be sent to all WHO Country Representative as well as Regional Offices for broad dissemination.
5. Gaps identified from the HPV DNA proficiency study should be followed up by taking appropriate corrective actions. Inclusion of an extraction control and clinical specimens is needed in future rounds of proficiency studies. For all HPV types included in mixtures of types in the proficiency studies, they should also be provided in the panel as a single type.
6. It is premature for the Global HPV Laboratory Manual to include descriptions of assays, before the assays have been assessed by at least several members of the WHO HPV LabNet; however, protocols for assays used in the LabNet may be posted on the LabNet website as information documents. The WHO HPV LabNet should provide some guidance on Quality Assurance and Control. Basic concepts in HPV testing should be included in the manual as precautions on the bench are important.
7. Samples for confirmatory testing should be sent from Regional Reference Labs to the Global Reference Labs. This could be extracted DNA.
8. Sampling methods are critical for HPV testing and alternative approaches should be explored.
9. A workplan for HPV surveillance data management should be developed.

#### **Recommendations on HPV Assays and Standardization**

1. Current HPV DNA testing is very expensive and is very critical for baseline surveillance before vaccination and for monitoring impact post vaccination. The WHO HPV LabNet is encouraged to develop "faster, cheaper and better" methodologies for global use.

2. LabNet members should develop or identify standardized assays for general use. These assays must be fully validated. This may start with evaluation and selection of appropriate assays through the proficiency study. Critical reagents must also be qualified before use. The supply of critical reagents for use in the development of a standardized DNA genotyping assay should be investigated.
3. WHO cannot recommend the use of specific HPV assays at the current stage. However both in-house assays and commercial assay kits may be evaluated by the WHO HPV LabNet in proficiency or collaborative studies.
4. Reagents such as qualified VLPs and monoclonal antibodies as well as quality control criteria for serological assays are urgently required. Further standardization efforts on VLP-ELISA is a high priority of the WHO HPV LabNet. Offers of commercially produced VLPs should be followed up.
5. Participants from WHO Regional Offices (AMRO, AFRO, EMRO, WPRO) expect standardized methodologies and standards for HPV testing to be available in order to expand the LabNet to national level through training programs.
6. Human cell DNA derived from C33a cells is suitable for use as a diluent of HPV DNA international standards. NIBSC should supply this when ampoules of the ISs are issued.
7. International standards should be developed for HPV DNA types 31, 33, 45, 52, 58 which are responsible for a significant number of cases of cervical cancer. Standards for currently available vaccine types 6 and 11 DNA and antibodies should also be considered for development.
8. International standards for antibodies should be from naturally infected individuals, independent of vaccines.

#### **Recommendations for WHO and public health authorities**

1. Public health authorities in different countries need to be informed of the aims of the HPV laboratory network so that they are aware of the possibilities of laboratory-based HPV surveillance and training for national labs not already in the HPV network.
2. Current LabNet members are largely based in academia, not public health, making it necessary to establish efficient communication with public health authorities. WHO's advocacy in helping to establish collaboration with public health officials is critical.

3. Gathering surveillance data is important for policy-making. The WHO strategic plan for the LabNet and WHO Recommendations on HPV surveillance and vaccination impact monitoring are urgently requested. Goals for HPV surveillance in each region should be defined. This will help the WHO HPV LabNet define the tasks to be undertaken at present and in the future.
4. There is a recommendation from the Reference Laboratories to set up WHO HPV LabNet focal points in each WHO Regional Office to disseminate information of the LabNet activities. This will be important for developing regional biennium workplan in the WHO Regions.
5. Funds at all levels are required for WHO HPV LabNet activities.

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## MEETING AGENDA

### Wednesday, 23 Jan 2008, WHO HPV LabNet meeting

9:00	Opening Remarks	I Knezevic
	Introduction	Participants
	Election of Chairperson and Rapporteur	
	Objectives of the meeting	TQ Zhou

- 9:30 Points to consider and proposed activities for WHO HPV LabNet, 2008-09  
TQ Zhou
- Discussion
- 10:00 Presentations from WHO HPV LabNet laboratories  
(presentation and discussion)
- progress review of 2006-07
  - proposed Terms of Reference for 2008-09
  - discussion and agreement & consensus reached on the TOR
- J Dillner (Global)
- 10:30 - 11:00 COFFEE BREAK
- 11:00 Presentations from WHO HPV LabNet laboratories (Continued)
- E Unger (Global)
- A Williamson (AFR)
- E Jerbi (EMR)
- D Haefliger (EUR)
- 12:30 - 14:00 LUNCH
- 14:00 Presentations from WHO HPV LabNet laboratories (Continued)
- S Sukvirach (SEAR)
- B Das (SEAR)
- K Kawana (WPR)
- S Garland (WPR)
- 15:30 - 16:00 COFFEE BREAK
- 16:00 Sustaining support to WHO HPV LabNet newsletter S Garland
- Discussion
- 16:30 Confirmatory testing/Proficiency Study in WHO HPV LabNet J Dillner
- Discussion
- 17:20 Plenary Discussion: Led by Chairperson
- How to build a functional WHO HPV LabNet?
- Revisit/Review of LabNet General TOR (2006), identify need for revision
- 18:00 Recommendations & Conclusions Chairperson
- Closure of the day

**Thursday, 24 Jan 2008**

9:00	Opening remarks	D Wood
	Self-introductions	Participants
	Election of Chairperson and Rapporteur	
	Objectives of the meeting	Chairperson
<b>Session 1</b>	<b>WHO strategic program in supporting HPV vaccine introduction</b>	
9:30	WHO strategic program in biological standardization	D Wood
	Discussion	
	WHO strategic plan in facilitating HPV vaccine development and introduction	
		K Irwin
	Discussion	
	Update on WHO global HPV LabNet progress	TQ Zhou
	Discussion	
10:30 - 11:00	COFFEE BREAK	
<b>Session 2</b>	<b>Review of current status in HPV testing areas towards standardization</b>	
11:00	Progress review and critical standardization issues in HPV DNA assays	
		E de Villiers
	Discussion	
	Progress review and critical standardization issues in HPV serology assays	
		M Ferguson
	Discussion	
	Study progress in characterization of the HPV antibody response	
		D Galloway
	Discussion	
	Experience and Challenges in HPV specimen collection and testing	
		LJ van Doorn
	Discussion	
12:10	Experience from industry	
	IFPMA	D Sikkema; M Deschamps
	DCVMN	J Shih
	Discussion	
12:45 - 14:00	LUNCH	

**Session 3 Progress in development of International Standards and potential needs for standardization**

- 14:00 Review of WHO/NIBSC ongoing/proposed standardization projects for HPV testing  
D Wilkinson  
Discussion
- 14:30 Implementation of International Standards in HPV laboratory testing  
Use of International Reference Reagent of anti-HPV 16 sera  
M Ferguson  
Discussion  
Report from a WHO Collaborative Study to Establish WHO International Standards for HPV 16 and 18 DNA Nucleic Acid Amplification Technology (NAT) Assays  
D Wilkinson  
Discussion  
Recommendations & Conclusions Chairperson and M Ferguson
- 15:30 - 16:00 COFFEE BREAK

**Session 4 Building the capacity of the global WHO HPV LabNet**

- 16:00 Views on harmonizing global HPV laboratory practice  
- from a Global HPV Reference Laboratory J Dillner  
Discussion
- 16:30 Perspectives from developing country settings  
- from a Regional HPV Reference Laboratory AL Williamson  
Discussion
- 17:00 Report of a WHO proficiency study for HPV DNA testing J Dillner  
Discussion
- 17:30 Close of the day

**Friday, 25 Jan 2008**

- 9:00 Proposed WHO HPV LabNet Workplan for 2008-2009 TQ Zhou  
Discussion  
Recommendations & Conclusions Chairperson & TQ Zhou

<b>Session 5</b>	<b>Role and function of WHO HPV LabNet in supporting global HPV vaccine introduction</b>	
9:30	Role and function of WHO HPV LabNet in HPV surveillance before and post vaccine	
	Introduction	E Unger
	Discussion	
	Developing a mechanism for HPV surveillance data collection and reporting in the WHO HPV	
	LabNet	E Unger & J Dillner
	Discussion	
10:30 - 11:00	COFFEE BREAK	
11:00	Perspectives and needs from regions in supporting HPV vaccine introduction	
	Briefing and discussion about HPV vaccine introduction/surveillance and expectations	
	AMRO	
	AFRO	
	EMRO	
	WPRO	
	Discussions	Led by E Unger & J Dillner
12:00	Recommendations & Conclusions	E Unger & J Dillner
12:30 - 14:00	LUNCH	
<b>Session 6</b>	<b>Review of Global HPV Laboratory Manual (the 1<sup>st</sup> Draft)</b>	
14:00	Presentation- Concepts and basic structure of the 1st draft of WHO HPV Laboratory Manual	
		J Dillner
	Review & Discussion	Led by J Dillner
15:30 - 16:00	COFFEE BREAK	
16:00	Action points/timeline for further revision of HPV Laboratory Manual	
		J Dillner
16:30	Closed session: Recommendations to WHO	Chairperson
17:30	Close of the meeting	