

WHO technical workshop on the role of laboratory detection of human papillomavirus in global disease prevention and control

Geneva, Switzerland, 15–17 August 2005

Immunization, Vaccines and Biologicals

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World Health
Organization

**The Department of Immunization, Vaccines and Biologicals
thanks the donors whose unspecified financial support
has made the production of this document possible.**

This document was produced by the
Initiative for Vaccine Research
of the Department of Immunization, Vaccines and Biologicals

Ordering code: WHO/IVB/06.04
Printed: April 2006

This publication is available on the Internet at:
www.who.int/vaccines-documents/

Copies may be requested from:
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Printed by the WHO Document Production Services, Geneva, Switzerland

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Abbreviations and acronyms

The acronyms listed below appear in this report.

CIN	cervical intraepithelial neoplasia
DNA	deoxyribonucleic acid
ECBS	Expert Committee on Biological Standardization
ELISA	enzyme-linked immunosorbent assay
GE	genome equivalent
GST	glutathione S-transferase
H	haemagglutinin
HAV	hepatitis A virus
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HPV	human papillomavirus
IgM	immunoglobulin M
IS	international standard
IU	international unit
N	neuraminidase
NAT	nucleic acid amplification test
NIBSC	National Institute for Biological Standards and Control (UK)
PATH	Program for Appropriate Technology for Health (USA)
PCR	polymerase chain reaction
PDZ	Psd-95, Dlg and Z01
QA	quality assurance
QC	quality control
RNA	ribonucleic acid
SARS	severe acute respiratory syndrome
SEAP	secreted alkaline phosphatase
SOP	standard operating procedure
VLP	virus-like particle

Summary

Acknowledging that human papillomavirus (HPV) is a common infectious virus with carcinogenic potential that is strongly associated with cancer development, especially with cancer of the cervix in chronically infected women, WHO convened a meeting of HPV experts to consider the role of the laboratory in the prevention of HPV-related cancers. The group reviewed the role that WHO-coordinated laboratory networks play in the context of other important infectious diseases, such as influenza, measles, pneumococcal infections, and poliomyelitis. For example, the influenza laboratory network has the task of conducting annual antigenic analysis on samples from patients with “influenza-like” symptoms, collected worldwide, in order to develop recommendations for the annual strain composition of influenza vaccines, and to assist in the adaptation of influenza vaccine formulation in preparedness for possible pandemics. The poliomyelitis laboratory network was set up as part of the poliomyelitis eradication initiative, and has a crucial role in ensuring that the initiative meets its objectives. The measles network provides expertise for the development and quality control of testing procedures, as well as accurate information for the measles mortality reduction and elimination initiative.

In the light of these WHO-coordinated laboratory networking activities, the HPV laboratory experts recommended the establishment of a global HPV laboratory network. The network’s mission would be to contribute to improving quality of laboratory services for effective surveillance and HPV vaccination impact monitoring, through enhanced, state-of-the-art laboratory support.

Initially, the proposed network would consist of a group of between 7–10 laboratories, including at least one HPV expert laboratory in each of the WHO regions, namely African, Americas, Eastern Mediterranean, European, South-East Asia, and Western Pacific Regions. These laboratories would need to fulfil quality criteria that were drafted by the expert group. Interested laboratories would be able to apply for network participation through the WHO web site, and applications would be reviewed by three independent experts (with no conflict of interest), appointed by the secretariat. The selected laboratories would undergo a site visit and, if appointed, would be asked to comply with the terms of reference, as drafted and agreed by the expert group, for a period of two years. Thereafter, reappointment would be subject to a biannual review of performance and renewal of engagement as a network laboratory. Subregional laboratories would represent the second step of the networking process and, finally, national/district/local laboratories would be able to join the network, following consultation with WHO Member States.

1. Introduction

Acknowledging that human papillomavirus (HPV) is a common infectious virus, with carcinogenic potential that is strongly associated with cancer development, especially with cancer of the cervix in infected women, WHO convened a meeting of HPV experts to consider the role of the laboratory in the global prevention of HPV-related cancers. Particularly in the light of recent advancements in HPV vaccine development and its anticipated introduction, the group was invited to focus on strategies to harmonize laboratory detection of HPV deoxyribonucleic acid (DNA) and antibodies. WHO has proposed a five-year plan to generate an enabling environment for HPV vaccine development and introduction, and has been awarded a grant, from the Bill & Melinda Gates Foundation, to implement the proposed efforts. The proposed plan includes three major objectives.

- a) Harmonize and standardize laboratory reagents and create an HPV laboratory network.
- b) Create an international multi-disciplinary policy platform and set a global agenda for future HPV vaccine introduction.
- c) Create a WHO 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 objectives of the meeting of HPV experts were to agree on establishing a global HPV laboratory network, to define a working structure, and to agree on the terms of reference for laboratories which would provide a programme for the dissemination of knowledge on accurate HPV testing. In addition, the group was to follow up on the recent progress made in the development of laboratory reference reagents and standards.

2. Background information

HPV infection plays a role in a variety of cancers, with cervical cancer accounting for 90% of these. The remainder include other genital cancers (5%), anal cancer (3%), and head and neck cancers (1%) (1). Clinical trials of HPV prophylactic vaccine candidates have been successful to date, and HPV DNA detection and antibody measurements have been central to characterization of the effects of vaccination (2).

Hence, accurate HPV DNA and antibody laboratory detection have proved crucial in epidemiological and vaccination studies, both for measurement of disease burden and vaccination outcomes. Laboratory studies allowed the association between HPV infection and cancer to be established, provided a molecular basis for classification of HPVs, and determined HPV type-specific prevalence in cancer cases and type-specific prevalence of infection in populations. This information was used to determine the antigen composition for development of vaccines likely to be effective globally. HPV type-specific DNA and antibody assays have also been key components to measuring vaccine efficacy in clinical trials, and will facilitate tests on the efficacy of novel vaccine candidates. In the near future, monitoring the serological levels of vaccine-induced antibodies may help determine the coverage of vaccination programmes in target populations in a post-vaccination period.

The HPV group of experts considered the role that laboratories play in the context of other important infectious diseases such as influenza, measles and poliomyelitis, as well as existing structures of WHO global laboratory networks for these vaccine-preventable diseases. It is envisaged that an HPV laboratory network would speed up the introduction of HPV vaccines by: facilitating the implementation of validated, standardized laboratory procedures; by developing quality assurance and proficiency testing; by training personnel and supplying equipment if required; and by providing a network for surveillance. While WHO will provide some support to initiate the network, the goal is to establish a structure that will allow for self-sustainability of a functioning HPV laboratory network.

2.1. Overview of WHO laboratory networks

2.1.1. *Influenza network: dynamics and synergies* (Dr M. Perdue)

In 1918, the so-called “Spanish influenza” killed about 50 million people worldwide. The observation that devastating influenza pandemics arise in a periodic manner has resulted in the establishment of the National Influenza Centres and Collaborating Centres (Influenza Network). The Influenza Network started in 1958 following the 1957 influenza pandemic (H2N2), with a goal to prevent another influenza pandemic. It is the oldest functioning WHO laboratory network, and conducts annual antigenic and genetic laboratory analyses on samples collected worldwide from patients with influenza-like illness. This monitoring is required because circulating influenza viruses in humans are subject to permanent antigenic changes, particularly in the haemagglutinin gene (H) “drift and shift”.

Influenza viruses are negative strand ribonucleic acid (RNA) viruses with a segmented genome. Through gene segment reassortment, they can display new combinations of antigens with major shifts in haemagglutinin (H) and neuraminidase (N) antigens, resulting potentially in a pandemic when immunologically naive populations are exposed to the new antigens. In birds, the viral infection is primarily enteric and viruses are shed in the environment, thus contributing to the spread of infection. In humans it is largely respiratory, although there is some enteric infection. Over the past 37 years there have been 17 antigenic events (10 in the last 2 years) which have been considered to have pandemic potential. Many experts accept that it is only a question of time until the next influenza pandemic occurs, and preparation is essential.

The mission of the influenza laboratory network is: to work towards reducing death from influenza by increasing global epidemic and pandemic preparedness; to conduct influenza surveillance by monitoring variation in the antigenic component and receptor-binding site of H (through sequencing); to measure burden of disease; and to develop phylogenetic trees for viral characterization and establishing patterns of spread (3). The network includes over 100 national laboratories that collect and characterize diagnostic specimens locally, seven WHO collaborating centres that perform sequencing and antigenic analysis and make recommendations for seasonal vaccines, and eight WHO H5-reference laboratories that characterize both human and animal isolates for pandemic vaccine development.

The most important role of the laboratories in the influenza network is to review data and provide recommendations on the dominating influenza virus(es) each year for vaccine composition/formulation to be used in manufacturing vaccines for the upcoming season, and to prepare for a possible pandemic. There is a WHO sequence initiative and a working group on polymerase chain reaction (PCR) primers to facilitate introduction of new tests, as well as a research network aimed at examining the human/animal interface. All network laboratories must be able to fulfil terms of reference and perform virus isolation and shipping according to WHO standards. WHO provides proficiency testing reagents and kits, and also training, but there is no additional financial contribution for the influenza network laboratories.

2.1.2. *The poliomyelitis laboratory accreditation model (Dr E. de Gourville)*

The poliomyelitis laboratory network (4) was set up in 1977 as part of the poliomyelitis eradication initiative, to implement the resolution of the Forty-first World Health Assembly on eradicating poliomyelitis worldwide (5). The laboratory network has a crucial role in ensuring that the initiative meets its objectives, particularly by means of systematic virological evaluation of suspected cases of acute flaccid paralysis for the presence of wild-type poliovirus in faecal specimens. The network provides: 1) validated standard laboratory procedures; 2) training for laboratory personnel; 3) standard reagents, controls and reference materials; 4) accreditation and proficiency testing; 5) infrastructure for data management; and 6) facilitation of international collaboration.

The 145 laboratories are organized into various levels of national, regional and specialized laboratories (6). The national laboratories generally serve their own country, to isolate and serotype viruses, to refer to reference laboratories, and to coordinate national surveillance and immunization personnel. Reference laboratories perform additional functions of differentiating polioviruses as wild or vaccine types, assist with training of personnel, and file evaluation of laboratory procedures. The specialized laboratories provide definitive confirmation of polioviruses by genome sequencing, and perform research on issues relevant to poliomyelitis eradication. WHO regional coordinators provide on-going performance monitoring of network laboratories, assist in problem solving, coordinate training, and assist in resource mobilization. Laboratory performance is monitored using established indicators and targets to evaluate sample collection and handling, timelines and accuracy of reporting. Laboratories take an annual proficiency test, and submit to an annual accreditation inspection, including on-site evaluation. A standard checklist is used to review the facility, its staff, proficiency test results, technical performance, biosafety, and data management. Accreditation is determined on the basis of standardized scores for between five and seven set criteria. Feedback is provided to the individual laboratory, the ministry of health, and international partners. The accreditation programme aims to improve laboratory performance, and the regional coordinator works with laboratories to address problems, as necessary.

2.1.3. *Measles laboratory network (Mr D. Featherstone)*

The measles laboratory network was developed along the lines of the successful global poliomyelitis laboratory network, utilizing much of the poliomyelitis laboratory network infrastructure (7). The WHO measles control goals differ for the six WHO regions, but have been largely established to achieve 90% reduction in mortality, due to measles, by 2010. The 702 measles network laboratories provide expertise for the development and quality control of testing procedures, and accurate information for the measles mortality reduction and elimination initiative (8). Laboratory testing is required for measles confirmation, as the case definition overlaps with other viral illnesses. In contrast to poliomyelitis, measles diagnosis is based on serologic assays (detection of specific immunoglobulin (IgM)). The network provides laboratory accreditation, standardized testing, and reporting. It has a tiered structure including specialized laboratories that maintain strain banks and establish quality assurance. WHO financial support is available and is dependent on the level of laboratory involvement in disease surveillance, contribution to the network, and gross domestic product of the country. The Centers for Disease Control and Prevention (USA) provide a major technical and financial partnership (9).

2.1.4. *Networking towards pneumococcal vaccine introduction* **(Dr T. Cherian)**

The pneumococcal laboratory network was only established recently to facilitate novel pneumococcal vaccine introduction. The laboratory network conducts epidemiological surveys to establish pneumococcus infection as a cause of severe childhood pneumonia, to determine incidence of severe pneumococcal disease, and to determine prevalent serotypes and antibiotic resistance patterns. The network is also involved in developing immunological assays and read-outs for vaccine evaluation, and in providing standardization of laboratory protocols, standard reagents, and training.

Once a vaccine is licensed, subsequent vaccines are evaluated on the basis of immunologic response. Experience has shown that different enzyme-linked immunosorbent assays (ELISA) give varied results. A set of 24 pairs of pre- and post- sera tested in 12 laboratories allowed improvement in the comparability of assay results. While antibodies are not established as immune correlates of protection, there is some degree of correlation between antibody levels induced by vaccination and the clinical protection against pneumococcal disease. Using this data, an antibody threshold could be identified to allow the establishment of markers for non-inferiority, and for comparison of different vaccine formulations.

2.2. Current understanding of HPV biology and laboratory safety

2.2.1. *State-of-the-art of HPV molecular biology* (Dr E. de Villiers)

Although about 20% of global cancers are linked to infections, the proportion varies greatly between developed and developing countries. Hepatitis B and C viruses are linked to liver cancers, Epstein-Barr virus to lymphomas and nasopharyngeal carcinomas, *Helicobacter pylori* is linked to gastric cancers, and human papillomavirus is linked to most cervical cancers (1). Of the infection-related cancers, 28% are related to HPV infection, and the burden of these cancers is greatest in women (52% as compared to 4% in males). HPV is the etiological agent of essentially all cervical cancer cases, about 50% of cancers of the vagina, about 25% of oral cavity, 70% of nail bed, and over 70% of anal cancers. For vulvar and penile cancers, the proportion related to HPV varies with histology, with over 50% of basaloid/warty types, and about 10% of keratinizing types.

HPV infection is extremely adapted to epithelial cells. Virus infection is initiated in basal epithelium and active replication is detected only in differentiated cells. The reported mean duration of persistence in the female genital tract is eight months, but about 30% of those infected remain positive at 12 months, with 9% still positive at 24 months. For oncogenesis to occur, three levels of host-specific cell signalling cascades must be breached: immune surveillance, intracellular regulation, and paracrine regulation of viral gene activity. Furthermore, physical and chemical carcinogens may also contribute to changes in these as well as other pathways. There is some effect of host genetic background as well. Therefore, HPV is necessary but is not sufficient for cervical cancer development. Global vaccination against HPV should therefore theoretically protect against HPV-related cancers.

2.2.2. *State-of-the-art of HPV antibody assays (Dr J. Schiller)*

There is currently no single “gold standard” for measuring the HPV serological responses, and different assays may be preferable depending on the questions asked. The types of assays include virus-like particles (VLP)-based ELISA assays, in vitro neutralization assays, and other sero assays. T-cell-based assays may not be appropriate to characterize responses of prophylactic vaccines, since antibodies are likely to be the critical effector molecules.

HPV VLP-based ELISA assays to the major viral capsid protein “L1” are type-restricted measures of present or past genital HPV infections. Their disadvantages are that high quality VLPs with intact conformation are difficult and expensive to produce, the conformation and quality may vary, and their sensitivity is only about 60% for prevalent infections (10). In addition, operating procedures and assay quality control have not been fully resolved and standardized. Approaches intended to improve the assay have been the inclusion of heparin to the ELISA plates, to capture properly folded L1 (11), or the use of an alternative antigen, such as the glutathione S-transferase (GST)-L1 fusion protein (12). The GST antigens are used in a multiplex bead assay and allow for the detection of antibodies against multiple types. However, characterization of the assay has been limited and 20% differences have been reported between the VLP and GST assays.

Previously available HPV neutralization assays were insensitive, cumbersome and/or time consuming. A surrogate assay relies on haemagglutination inhibition. It is based on the observation that VLPs crosslink mouse red-blood cells. In the presence of an inhibiting L1-specific antibody, this agglutination is abolished or reduced. The disadvantage of the haemagglutination inhibition assay is that only a subset of neutralizing antibodies prevents agglutination. A similar limitation applies in the case of competitive radioimmunoassay, as only a subset of antibodies is able to compete in the assay.

Recently, a simple and high throughput pseudovirus-based in vitro neutralization assay was developed (13). Neutralization is monitored by measuring the activity of secreted alkaline phosphatase (SEAP), which is expressed from a SEAP gene that is included in the pseudovirus as a reporter gene. The approach is easy to scale up, as pseudovirus stocks are not a limiting part of the assay. Chemiluminescent detection improves the dynamic range of the assay sensitivity. The SEAP neutralization and VLP-ELISA assays have comparable sensitivity for detecting capsid antibodies generated after natural HPV infections. However, the SEAP assay is substantially more specific in detecting type-restricted antibody responses to VLP vaccination, and available data suggest good inter-laboratory reproducibility. The limitations of SEAP neutralization assay are that there may be non-specific inhibition at low sera-dilutions, it requires cell culture, and chemiluminescent reagents are expensive.

In conclusion, for assessment of cumulative exposure to genital HPV infection prior to vaccination, the preferred assay to use may be the VLP-ELISA, since it is the best validated assay, and is easier and less expensive to conduct than the SEAP pseudovirus assay. However, the availability of high quality VLPs may be a limiting factor. The SEAP assay measures all classes of antibody relevant to protection, is type-restricted in measuring VLP-induced antibodies, and is relatively simple with high throughput, with the result that it could be used to monitor vaccine performance in central laboratories, or locally after shipment/storage of sera. It might be particularly useful in the attempt to study immune correlates of protection.

2.2.3. Biosafety considerations (Dr N. Previsani)

Biosafety is defined as containment principles, technologies and practices implemented to prevent unintentional exposures to pathogens and toxins or their accidental release. WHO Member States recently passed a resolution on enhancement of laboratory biosafety (14). WHO is thus taking an active role in improving biosafety, providing technical support, and reporting to its Executive Board on progress towards implementation of the resolution. Safe practices addressed include those within the laboratory, transportation and field investigation, and in manufacturing facilities. Recent public health problems, such as the laboratory-based outbreak of severe acute respiratory syndrome (SARS), have highlighted the need for laboratory safety. WHO visited those sites where laboratory-acquired SARS infection was reported, and made recommendations aimed at resolving the problems. Safe practices in health-care facilities fall under a different category and are dealt with by a different group within WHO.

The third edition of the *Laboratory Biosafety Manual*, which is used for laboratory commissioning and certification by WHO, has just become available on the WHO website (15). International regulations for transportation of biological materials are decided at the United Nations level for each mode of transportation, and the International Civil Aviation Organization formulates air transportation regulations that are translated by the International Air Transport Association into specific rules for the shipment by air of biological materials.

2.3. Role of the laboratory in management and control of HPV diseases

2.3.1. Relationship between analytical and clinical sensitivity of HPV testing (Dr C. Meijer)

Given the fact that persistent infection with high-risk HPV is causally involved in cervical cancer, the question arises as to the relationship between the presence of cervical intraepithelial lesions and HPV viral load. Because of the implications in terms of diagnostic algorithms and screening programmes, a study was initiated to explore this relationship, i.e. to determine HPV viral load measurements in cases of normal cytology and cervical intraepithelial neoplasia (CINs) (16). Indeed, some detection methods with very high analytical sensitivity described in the literature will show a higher positivity rate in women who do not have high risk for CIN development and cancer. These tests result in unnecessary followup, which in turn may increase related health-care costs. Thus, high analytical sensitivity tests are useful in research and monitoring settings rather than in clinical management.

2.3.2. Development of novel technology (Dr J. Sellors)

In view of the role HPV infections play in cancer development, HPV DNA testing represents a potentially effective tool for screening, but it requires an affordable test. Cost effectiveness is largely dependent upon coverage, but numbers of visits, level of service (i.e. local versus specialist clinic), frequency of screening, age, and test accuracy and reproducibility are also important components of cost. The Program for Appropriate Technology for Health (PATH) USA, has initiated a project for the development of two distinct rapid HPV tests for screening in low resource settings. For this project PATH has two commercial partners, which are each developing their own test using different technology platforms for detection.

The test under development by Digene Corporation (Gaithersburg, MD, USA) detects HPV DNA, and processes 10–100 samples in one batch within two hours. A chemiluminescent substrate is used and signal output is read by visual inspection of a Polaroid film exposed to the reaction. Luminometer readout is also possible at a central reference laboratory. The sensitivity of this test is comparable to the currently available Hybrid Capture 2, and may be most relevant for clinical referral. On the other hand, Arbor Vita Corporation (Sunnyvale, CA, USA) is developing a 15-minute, point-of-care, one-at-a-time rapid strip test that is based on detection of an HPV oncogenic protein as biomarker (E6) which is expressed in CIN3 lesions. The E6 detection assay is based on a lateral flow platform and on the observation that E6 proteins of oncogenic HPVs bind to Psc-95, Dlg and Z01 (PDZ) proteins (17). The binding is strong for E6 proteins from oncogenic types as opposed to non-oncogenic types (18). The value of detecting oncogenic E6 HPV proteins in vaccination studies has not been explored. Evaluation of the feasibility of rapid tests and their value in vaccination settings has also not been studied.

2.4. Quality assurance and standards

2.4.1 Quality standards for vaccines and biologicals (Dr D. Wood)

Quality standards for vaccines and biologicals are supported by three products produced by WHO, including: (a) global written standards: WHO expert committee reviews proposed standards and guidelines published as the report series (19); (b) global measurement standards: standard reagents to validate assays; and (c) technical support for science-based evidence to assist with (a) and (b).

- a) Written standards are guidelines that outline technical specifications to define the safety and efficacy of vaccines and biologicals. The reports are scientific and advisory in nature. Guidance is provided to national regulatory authorities and manufacturers for good manufacturing practice, as well as clinical and non-clinical evaluation, and for facilitation of international harmonization of vaccine licensure. WHO requirements (in general guidance documents) are applicable to most virus vaccines. Examples include cell substrates (*WHO Technical Report Series*, No. 878), clinical trials (*WHO Technical Report Series*, No. 924) and non-clinical guidelines (*WHO Technical Report Series*, No. 926). The written standards are not regulatory but do provide recommendations on production and quality control (QC) for vaccines, and serve as a basis for national policy.
- b) Measurements standards or reagents serve to provide a comparison of performance between laboratories. The standards serve to harmonize international regulation and assist in vaccine, diagnostic and therapeutic development. Specifications for preparation, development and establishment of WHO standards are described in *WHO Technical Report Series* No. 932 (in press). Development of an international standard is followed by the development of secondary data that is then distributed to regulatory authorities, manufacturers and product users. Examples of standards include human immunodeficiency virus (HIV)-1 genotype reference panel, hepatitis A virus (HAV), hepatitis B virus (HBV) and hepatitis C virus (HCV). The effect of standards is exemplified in the case of plasma-derived products, whereby technical specifications for assays verifying that products are free of these viruses can be set by various regulatory authorities.

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- c) Support for science-based evidence to develop vaccines. This objective is achieved through: (a) convening meetings of appropriate experts in the field, covering both scientific and technical expertise, such as on immune correlates of protection; and (b) global communication of consensus advice to all stakeholders, including development of guidelines based upon scientific rationale, evidence for efficacy trials, immunological memory, protection in animal models, and evaluation of T-cell responses.

The process of setting standards entails consultation between all stakeholders including regulators, manufacturers, and researchers. This leads to draft guidelines, as in the case of international standard (IS), collaborative studies, and ultimately established standards.

2.4.2. Preparation of HPV DNA standard reagents (Dr M. Ferguson)

International standards for nucleic acid amplification tests (NATs) have been prepared for key viruses that are important in blood safety — hepatitis A, hepatitis B, hepatitis C, HIV-1, and parvovirus B-19. Assays are validated using these standards prior to their routine use. Negative results for these agents in plasma pools are required prior to release of blood products within Europe. The availability of standards also allows the various NAT assays used in testing of these products to be compared, to ensure that they have the required sensitivity. A negative result thereby assures the safety of a product. Inclusion of a working standard or “run-controls” (positive and negative) in every assay allows laboratories to monitor their performance and improve testing results. For example, over the course of several months, one laboratory’s performance gradually improved from no detection of a 1:10 dilution of HCV ribonucleic acid (RNA) working standard, to 100% detection. Another study showed that standards allow laboratories to establish the acceptable level of parvovirus B19 in blood products at 10^4 international units (IU)/ml, to permit their use and harmonize laboratory testing.

A biological material is a complex substance that cannot be fully characterized by physical/chemical means and thus requires some sort of bioassay. Reference materials for biologicals are therefore calibrated in arbitrary rather than absolute units, with the units referring to the activity of the analyte. Reference or standard reagents allow results of different assays to be compared. The availability of international standard materials facilitates interlaboratory comparisons and improves laboratory performance.

An international standard is a preparation to which an international unit (IU) of activity has been assigned. Local or working standards are prepared from national standards or from international standards. Plasma containing infectious virus was used to prepare the HCV and HBV standards, whereas the HPV community has a history of using HPV plasmid preparations to determine genome equivalents based on DNA concentration. On the other hand, determination of genome equivalents from viral-extracted DNA requires at least one enzyme to copy, and oligonucleotides to hybridize, and target sequences and methods are never 100% effective.

Making the comparison to genome equivalents (GE) may be misleading where methods are complex, and results may be assay-specific. For HPV DNA detection, the National Institute for Biological Standards and Control (NIBSC, UK) will prepare the proposed candidate standards based on plasmids containing HPV full genomic cDNA sequences, in the presence of C33A cellular DNA matrix, as previously agreed (20). This is the same material that was evaluated in an international collaborative study (21). Plasmids will be diluted to contain 10^7 copies (GE/ml). The fill volume of ampoules will be not less than 0.5 ml to ensure satisfactory precision, and a total of 5000 ampoules prepared. Preliminary studies are being performed to verify material stability and reliability. These include titration of the plasmid in trehalose, as trehalose has been used as an excipient (stabilizer) in some candidate DNA preparations, as well as a trial to establish stability of the plasmid during the freeze-dry process. Laboratories involved in the first collaborative study will be invited to perform at least three independent assays, conducted one week apart. In the first assay, ten-fold dilutions will be used to determine the endpoint of detection. Subsequently five half-log dilutions will be used on either side of the endpoint to refine dilution endpoint. A single PCR will be used for each dilution. All samples in dilution series are tested concurrently using the diluent normally used in the assay. Results will be returned to NIBSC for statistical analysis. Laboratories will then prepare in-house standards run against IU to be used as run-controls in every assay.

During the HPV group discussion, a number of the laboratory experts suggested that the standard should be assigned a unitage in genome equivalents or copy numbers as determined by best physical/chemical methods. They suggested that the HPV community would accept such a definition more readily. It was also agreed that the HPV DNA standard would not be used as a control for the extraction procedure, as purified DNA will be used for the standard material. Therefore it should be understood that the only parts of the assay addressed to date in international collaborative studies are amplification and detection. Establishment of standard operating procedures (SOPs) will also contribute to worldwide harmonization of HPV assays.

2.4.3. Preparation of HPV 16 antibody standard reagents and proposed studies (Dr M. Ferguson)

An international standard reagent is a preparation to which an international unit of activity has been assigned. Such standards are intended for use in the estimation of potency of an appropriate test sample by direct comparison in suitable biological test systems. International standards are established by the WHO Expert Committee on Biological Standardization (ECBS) (22).

Candidate international standards (sera) for viral antibodies, are evaluated in collaborative studies to characterize them in terms of reactivity in the range of assays typically performed in different laboratories. In addition, the studies serve to assess commutability, i.e. to establish the extent to which the reference standard is suitable to serve as a standard for a variety of different samples being assayed.

Generally, a series of dilutions of the standard sera, and test samples are assayed. Data are analysed by standard statistical analysis programmes, probit analysis or parallel line analysis. The antibody concentration or potency of other study samples is expressed relative to the standard. The range of dilutions tested is taken into consideration in this analysis. If the standard has an assigned unitage of 50 IU/ampoule, the relative potency is multiplied by 50 to express the antibody concentration of the test sample in IU.

A pilot study on assays for anti-HPV 6, 11, 16, and 18 antibodies has been conducted. The objective of the collaborative study was to characterize the standard for reactivity as well as commutability, i.e. if the sample could be used in both natural infections and vaccine evaluations. The study assessed the performance of different laboratories on a panel that included serological samples derived from virgins, from individuals known to be naturally infected, and from HPV-VLP vaccine recipients. The assays that were used included neutralization of pseudovirus and VLP-based immunoassays. Expression of results as antibody concentration related to a reference sample, showed a closer fit among laboratories and assays and appeared to be consistent across laboratories. Results showed that there was a good correlation between HPV 16 neutralization and immunoassays related to the range of titres, and the reactive samples were ranked consistently by each laboratory as to potency relative to the study reference sample (23).

A candidate standard pooled-sera for HPV 16 antibodies, made from sera pooled from naturally infected subjects, has been prepared by NIBSC. The suitability of this material as an international standard will be assessed in a second collaborative study. Additional coded samples which may be included are:

- duplicate ampoule of the candidate standard
- an HPV 16 serum from a naturally infected individual
- an HPV 18 serum from a naturally infected individual
- a negative serum
- sera from recipients of HPV vaccines close to licensure.

Participants will be asked to use the methods in routine use in their respective laboratories to perform three independent assays (preferably one week apart), to prepare and test a series of dilutions from the candidate standard and each of the coded samples. This should include all study samples in each assay so that the concentration of antibodies relative to the candidate standard can be calculated. They should also submit raw data from assays to NIBSC, and report each sample as positive or negative, based on their calculated cut-off value. The study was scheduled to start in October 2005 and finish by May 2006. The results on the validation of antibody reagents will be submitted to ECBS for approval as international standard reagents to HPV 16 antibodies.

3. Developing a global HPV laboratory network

WHO has successfully established laboratory networks to achieve specific missions. The influenza laboratory network's mission is prevention of a pandemic; the measles network's mission is elimination or disease reduction; and the poliomyelitis network's mission is eradication. In view of these WHO-coordinated laboratory networking activities, the group of HPV laboratory experts recommended the establishment of a global HPV laboratory network, to support the introduction of HPV vaccines, and surveillance of disease and infection. Furthermore, it was recognized that support for clinical diagnosis or screening could run concurrently with this effort but should not be the major focus of the network at this stage.

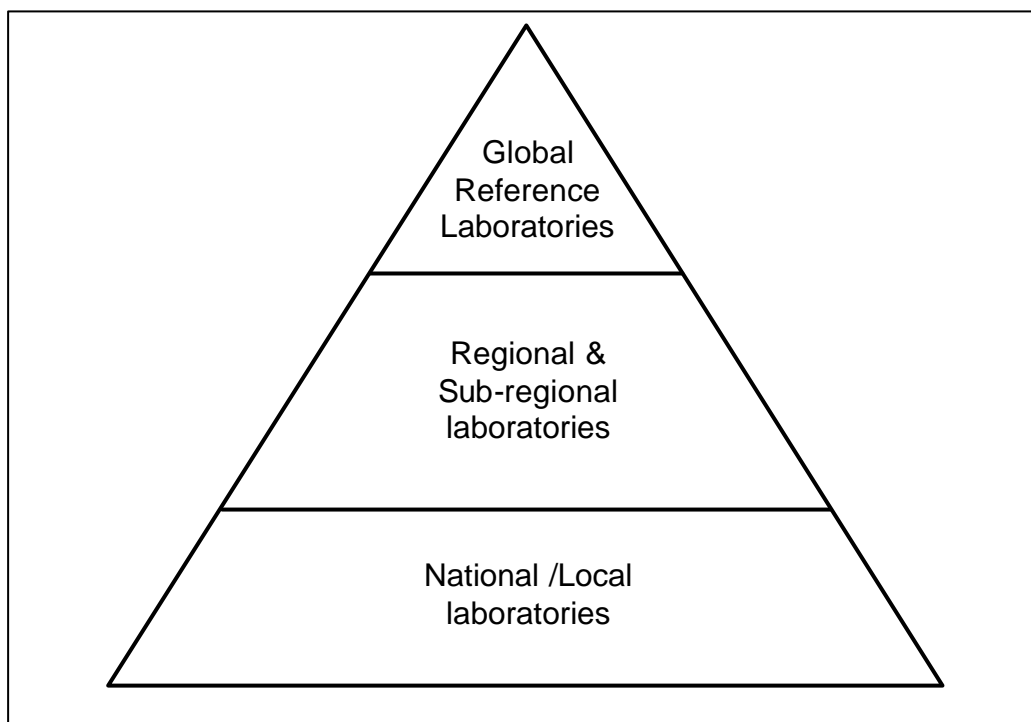
Laboratory harmonization and standardization of methods has started with international collaborative studies on HPV DNA and antibody detection. The general terms of reference for laboratories to join the HPV network were drafted and agreed upon.

3.1. Terms of reference

The proposed mission for the global HPV laboratory network would be to contribute to improving quality of laboratory services for effective surveillance and HPV vaccination impact monitoring, through enhanced, state-of-the-art laboratory support.

Expert laboratories will be identified to support HPV-laboratory work, and will facilitate public health strategies for HPV-vaccination. The network structure will comprise laboratories at three levels of involvement: reference, regional and subregional.

Figure 1: Network structure



Laboratories are expected to assume national, regional and/or international responsibilities, and to be instrumental in developing and supporting HPV laboratory work in their respective geographical areas.

In its respective capacity, each laboratory would be expected to be active in the following four areas:

- Scientific and technical advice
 - Provide scientific advice to the HPV laboratory network in its region, in virological and serological surveillance of HPV infections (reference and regional levels).
 - Collaborate with local and regional public health and research institutions, as well as with WHO and other international agencies, on monitoring HPV vaccination (all levels).
 - Disseminate knowledge on, and the use of, HPV international standard reagents to improve accuracy of genotyping, and serological measurements and derived information (all levels).

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- Quality assurance
 - Participate in developing guidelines and SOPs for establishing a regional laboratory-based quality control programme (reference and regional levels).
 - Serve as a resource for storage and distribution of standardized reagents, proficiency panels and cell lines to other laboratories as required (reference and regional levels).
 - Ensure that all HPV assays perform at acceptable levels of sensitivity, specificity and reproducibility (all levels). Critical test reagents used in WHO studies should be validated by the relevant WHO reference laboratory prior to utilization.
 - Participate in on-site visits to other countries/provinces as part of the WHO evaluation team, if requested (all levels).
 - Perform confirmatory testing on samples from other laboratories in the project area, if necessary (reference level).

 - Training
 - Contribute to developing training materials for HPV laboratory research and surveillance within its respective region, as required (all levels).
 - Coordinate and participate in WHO laboratory training workshops for staff within the laboratory network, as required by WHO (all levels).
 - Assure that sufficiently trained and qualified personnel are available to fulfill the tasks related to HPV detection and serology (all levels).
 - Provide training on the appropriate collection of clinical samples for HPV typing (all levels).

 - Communication
 - Promote and participate in the exchange of information between national, regional and reference laboratories, and the HPV laboratory network (all levels)
 - In consultation with the WHO laboratory focal point, raise funds for specific activities related to the network (all levels).
 - Within determined timelines, provide information to WHO on laboratory activities, and an annual compilation of virological and serological surveillance (all levels).

The HPV laboratory network will ensure the availability of competent laboratory services worldwide. Its structure will be based on three responsibility levels which will be assumed voluntarily by institutions included in the network, namely: global reference laboratories; regional and sub-regional laboratories; and national/local laboratories. Initially at least one HPV regional laboratory for each continent/WHO region would be established, namely African, Americas, South-East Asia, European, Eastern Mediterranean, and Western Pacific (24).

3.2. Requirements and accreditation criteria for network laboratories

Accreditation will provide documentation that the laboratory has the capacity and the ability to detect, identify type, and report human papillomaviruses that may be present in epidemiological surveillance and clinical samples.

The activities of the **global reference laboratories** would be to:

- provide technology platforms and tools for surveillance to define the circulating HPV types and relationship to vaccine design;
- provide definitive identification and typing of HPVs isolated, using all available technologies, including sequencing, to ascertain the variant of the isolates;
- contribute to the preparation and distribution of relevant standard reagents and training materials to regional laboratories;
- contribute to design, preparation, and validation of proficiency panels which would ensure that all HPV testing (virological and/or serological) was performed at acceptable levels of sensitivity, specificity and reproducibility;
- provide specialized training and trouble-shooting assistance to regional laboratories;
- provide confirmatory testing on samples as required;
- participate in developing WHO guidelines and SOPs for regional laboratories;
- participate in on-site visits to other countries/provinces as part of the WHO evaluation team if required;
- participate in research aimed at improving the sensitivity, specificity, applicability and speed of methods and procedures for the detection of HPV DNA and antibodies;
- report results in a timely manner;
- coordinate activities with the WHO HPV laboratory network.

Tasks of **regional and subregional laboratories** would be to:

- provide information on the use of international standard reagents for HPV DNA and antibody detection to national laboratories within their geographical region;
- serve as training centres as required, particularly on issues of laboratory biosafety;
- manage quality assurance/proficiency programmes within the region;
- participate in global quality-assurance programmes;
- participate in sample collection, detection, and identification of human papillomaviruse types that may be present in survey and clinical samples;
- refer selected HPV isolates to reference laboratories for sequencing;
- coordinate with WHO regional offices;
- report results and other information regarding HPV within the region to WHO regional offices.

Tasks of **national/local laboratories** would be to:

- provide assistance as required on transferring laboratory technology, methodology and protocols regarding HPV DNA and antibody detection to other laboratories within their country;
- provide information on the use of international standard reagents for HPV DNA and antibody detection;
- participate in quality assurance programme;
- comply with biosafety guidelines;
- refer selected HPV isolates to reference laboratories for sequencing;
- coordinate with national authorities on all laboratory-based information relating to HPV diseases;
- report results in a timely manner.

3.3. Tasks related to communication

WHO would provide information concerning the HPV laboratory network on the WHO web site, via the appropriate web page and links. This would include information on scientific meetings and training courses. Annual reports would not be published on the web. Reference and regional laboratories would be expected to maintain close contact with each other, and a regular exchange of information (e-mail) would be required.

Laboratory network meetings would be necessary, and annual meetings are recommended for the initial phase, until the network is established. Longer intervals (biannual) may be sufficient in the later phase.

3.4. Tasks related to fundraising

The general consensus was that initial financial support would be required for the reference and regional laboratories, primarily for reagents and consumables, and if necessary, also for staff and equipment. It was agreed that the WHO secretariat would provide support for network activity fundraising, respond to applications, and would coordinate support for laboratory network meetings within the scientific community/organizations, particularly those involved in laboratory work. First attempts would be made within the European region, and thereafter within the regions of the Americas and Asia.

3.5. Network training activities

It was agreed that for a functioning network the laboratories should have involvement in public health, and should preferably be able to provide evidence of efforts in improving it. Reference laboratories would offer training courses (theoretical and practical) for staff members of regional laboratories. Laboratories should have experts in virology, be internationally recognized, and should have produced publications in the relevant area. Preferably, laboratories should also have had both HPV DNA as well as specific antibody experience. Diagnostic routine work must be a separate function, and can be allowed, provided other laboratory obligations are fulfilled. Testing performed as part of network activities should not be used for clinical diagnosis.

Training recommendations made were to prepare an envisioned HPV laboratory training manual on combined “Managerial” and “Technical” issues following the outline:

- introduction to HPV and relation to clinical manifestations;
- background on basis of laboratory methods for HPV;
- basis of vaccination and relevance to public health;
- specimen collection, storage and processing;
- recommendations for “standard” specimen collection with options for a variety of acceptable options (for cervix), (no recommendation for sampling of men at the initial stage).
- HPV DNA assays:
 - SOPs (including use of international standards and in-house standards/calibrants)
 - QA/QC methods
 - data interpretation
 - troubleshooting.
- HPV serology assays:
 - SOPs (including use of international standards and in-house standards/calibrants)
 - QA/QC methods
 - data interpretation
 - troubleshooting.
- Validation of assays on site.
- Data management.
- Biosafety.
- Guide to providing training to others:
 - needs assessment
 - networking to provide ongoing training and supervision.

It was agreed that DNA testing capacity is needed now to establish prevalence data, while serology capacity will be needed when vaccine studies are initiated in a given region. An expert group will review and prioritize training.

3.6. Biosafety recommendations

Laboratories working with HPV should follow WHO Biosafety Guidelines, level 2:

- use gloves, work under a hood, incinerate waste;
- prevent environmental contamination – potential for other infectious agents such as HBV and HIV at least as great a risk as HPV;
- handle biological samples as if potentially infected so no need to test for HIV, etc.

There are no additional requirements for HPV.

There is no need for the statement “HPVs are carcinogenic, and hazardous for human beings”, nor for pipettes or bench to be labelled “HPV only”. There would also be no need to monitor staff for contamination.

3.7. Standard operating procedures

Recommended detection methods should allow improvements in HPV DNA genotyping for all laboratories in the network.

- Consensus PCR with type-specific hybridization favoured current format.

Expectations for each laboratory’s sensitivity and specificity should be based on assay, and:

- no differentiation by laboratory hierarchy;
- international standard reagents used to set thresholds;
- clinically significant thresholds favoured as minimum.

HPV antibody assays should use minimum HPV 16 and HPV 18 VLPs.

- WHO should consider procuring monoclonal antibodies to permit laboratories to evaluate VLP quality.
- SOPs for neutralization assays and VLP production will be very difficult to implement. Coordinated procurement for VLPs will therefore be explored.

All SOPs need to be field-tested to demonstrate effective implementation, including:

- specimen processing and storage, with information on collection methods;
- equipment maintenance;
- quality assessment of VLP and other reagents prepared in-house.

3.8. Proposed implementation timeline

Interested laboratories can apply for network participation through the WHO website. Applications will be reviewed by three independent experts (with no conflict of interest) appointed by the secretariat. The selected laboratories will undergo a site visit and, if appointed, will be asked to comply with the terms of reference, as drafted and agreed by the expert group, for a period of two years. Reappointment will be subject to biannual accreditation. Sub-regional laboratories will represent the second step of the networking process, and finally, following agreement with the regional laboratories, national, district, and local laboratories will be able to join the network.

The HPV expert group agreed that reference laboratories will be identified and expected to serve the laboratory network. It was also agreed that criteria to identify and select between five and seven regional expert laboratories for DNA testing will be displayed on the WHO web page. Applications will be reviewed by an expert panel, and appointments will be made by WHO within six months. Regional laboratories will be expected to invite other laboratories in the same region to participate. Initial work will focus in areas where vaccines are likely to be implemented.

4. Conclusions

A mission statement was adopted for the network: “To contribute to improving quality of laboratory services for effective surveillance and monitoring of HPV vaccination impact, through enhanced, state-of-the-art laboratory support.”

WHO’s agreed role in the network would be to provide coordination through a focal point in WHO headquarters who would liaise with laboratory-responsible staff in each regional office, as well as with network members, as appropriate. WHO would also monitor the benefit and progress of the network through established indicators, such as:

- adoption of international standards
- availability of HPV laboratory manual
- implementation of training programmes.

In addition, WHO would help allocate resources for network functioning, as appropriate, would coordinate reports and network meetings, and would assist in fundraising.

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The World Health Organization has managed cooperation with its Member States and provided technical support in the field of vaccine-preventable diseases since 1975. In 2003, the office carrying out this function was renamed the WHO Department of Immunization, Vaccines and Biologicals.

The Department's goal is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. Work towards this goal can be visualized as occurring along a continuum. The range of activities spans from research, development and evaluation of vaccines to implementation and evaluation of immunization programmes in countries.

WHO facilitates and coordinates research and development on new vaccines and immunization-related technologies for viral, bacterial and parasitic diseases. Existing life-saving vaccines are further improved and new vaccines targeted at public health crises, such as HIV/AIDS and SARS, are discovered and tested (*Initiative for Vaccine Research*).

The quality and safety of vaccines and other biological medicines is ensured through the development and establishment of global norms and standards (*Quality Assurance and Safety of Biologicals*).

The evaluation of the impact of vaccine-preventable diseases informs decisions to introduce new vaccines. Optimal strategies and activities for reducing morbidity and mortality through the use of vaccines are implemented (*Vaccine Assessment and Monitoring*).

Efforts are directed towards reducing financial and technical barriers to the introduction of new and established vaccines and immunization-related technologies (*Access to Technologies*).

Under the guidance of its Member States, WHO, in conjunction with outside world experts, develops and promotes policies and strategies to maximize the use and delivery of vaccines of public health importance. Countries are supported so that they acquire the technical and managerial skills, competence and infrastructure needed to achieve disease control and/or elimination and eradication objectives (*Expanded Programme on Immunization*).

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