



WHO HPV LabNet - Newsletter 01

WHO HPV LabNet: World Health Organization's Global Human Papillomavirus (HPV) Laboratory Network



Preface: This newsletter aims to provide brief and updated information about the WHO HPV LabNet activities. It will be produced on a 6 monthly basis, *this being the first issue*.

- Content:**
1. Background of the WHO HPV LabNet
 2. Participating laboratories in the HPV LabNet
 3. Establishment of International Standards for HPV detection
 4. Recent / Upcoming Meetings
 5. Projects Ongoing
 6. References
 7. Useful Web Links

1. BACKGROUND OF THE WHO HPV LabNet

As a consequence of the recent advances in HPV vaccine development, HPV experts in unison have recognized the need for harmonization of HPV laboratory assays at the outset of the development and implementation of new prophylactic HPV vaccines.

(<http://www.who.int/vaccines-documents/DocsPDF06/845.pdf>).

Mission of the HPV LabNet is:

"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".

It is envisaged that the HPV LabNet would speed up the introduction of HPV vaccines by: facilitating the implementation of validated, standardized laboratory procedures; by developing quality assurance system and proficiency testing; by training personnel and supplying equipment if required; and by providing a network for surveillance.

The HPV LabNet will ensure the availability of competent laboratory services worldwide for the evaluation of HPV DNA and antibody detection in biological specimens through capacity-building and strengthening for those who are in need by providing up-to-date technical information, training on laboratory practice, technical advice and guidance.

Structure of the HPV LabNet - is based on three levels of responsibility assumed voluntarily by institutions, which are subject to independent expert review and site visit led by WHO; namely:

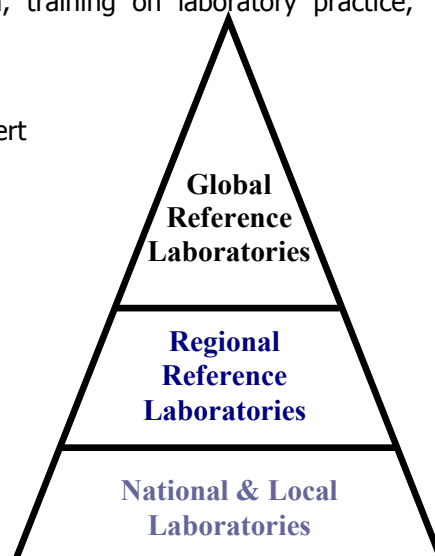
- Global reference laboratories,
- Regional laboratories, and
- National/local laboratories.

Tasks of the HPV LabNet - are actively conducted in the areas of:

- Scientific and technical advice,
- Quality assurance,
- Training, and
- Communication.

Specific tasks are defined for each reference laboratory based on the expertise and capacity. More services are provided at the Global HPV Reference Laboratories such as confirmatory testing of samples from regions/countries.

More details are found at: http://www.who.int/vaccine_research/diseases/hpv/TORHPVlabnetfeb06.pdf.



2. PARTICIPATING LABORATORIES IN THE HPV LabNet (September, 2007)

GLOBAL WHO HPV REFERENCE LABORATORIES

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*Calls for application to the
National HPV Reference
Laboratories are yet to be
opened.*

WHO COLLABORATING CENTRE FOR BIOLOGICAL STANDARDS

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3. ESTABLISHMENT OF INTERNATIONAL STANDARDS FOR HPV DETECTION

- WHO has a major role in the establishment of International Standards for biological material and reference reagents used in prophylaxis, therapy and/or diagnosis of various human diseases (http://www.who.int/biologicals/reference_preparations/en/).
- Establishment of international reference reagents for HPV should facilitate inter-laboratory comparisons and detection worldwide.
- With the support of WHO, two international collaborative studies have been completed with the **primary aim of establishing candidate International Standards for HPV**. In addition, it was intended to assess inter-laboratory variability in HPV testing methods and outcomes for HPV antibody assays and HPV type-specific DNA.

3.1 First WHO international collaborative study on the standardization of the detection of antibodies to HPV ^{1, 2}

Objectives:

- Compare the performance of HPV capsid-specific antibody assays in use by **10 laboratories** worldwide.
- Determine participating laboratories ability to perform:
 - Correct identification of HPV antibody and its genotype specificity,
 - Ranking of sera by titre, and
 - Correct detection of HPV16 and HPV18 in presence of other HPV antibody types.
- Investigate the expression of HPV16 antibody levels relative to a HPV16-infected subject as a standard, to determine whether establishment of an International Standard for HPV16 antibodies would facilitate comparison of such measurements between laboratories and assays.

Methods:

- 12 identical serum samples were distributed to 10 laboratories:

- Naturally infected with HPV (HPV 6/11, 16, 18, or all 4 types) ($n=6$),
- Vaccinated with monovalent HPV 16 virus-like particles ($n=5$),
- Non-sexually active ($n=1$),
- Laboratories were requested to test these samples using methods in routine use.

Table 1 Printed with permission ²

REACTIVITY FOR ANTIBODIES TO HPV 16 AS DETERMINED IN A NEUTRALIZATION AND IMMUNOASSAYS			
Sample		Reciprocal of end point dilution in neutralization tests	Range of titers for HPV 16
01	HPV 16 natural infection	100	100–640
08	HPV 16 natural infection ¹	40	<20–160
09	Natural HPV 6+11+16+18	30	<20–80
03 ¹	Pool HPV 6+11+16+18	20	<100–640
13	HPV 18 natural infection ¹	<20	Negative
16	HPV 6+11 natural infection ¹	<20	Negative
12	Negative	<20	Negative
04	Vaccine	1,600	100–2,560
05	Vaccine	400	384–2,560
07	Vaccine	5,445	400–2,560
10	Vaccine	10,240	1,600–10,240
14	Vaccine	87,040	5,381–40,960

Results:

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TABLE 2 – HPV-16: POTENCY RELATIVE TO SAMPLE 01 WHICH WAS ASSIGNED A UNITAGE OF 1.0 UNITS											
Sample		Laboratory									
		1	2	3	4	5	6	7	8	9	10 ¹
08	HPV 16 natural infection	0.24	0.45	0.37	0.37	0.54	0.50	0.42	0.40	0.68	0.74
09	Natural HPV 6+11+16+18	0.09	0.54	0.41	0.27	0.36	0.29	0.16	0.10	–	0.30
03	Pool HPV 6+11+16+18	0.07	1.10	0.20	0.22	0.29	0.26	0.11	0.25	0.19	0.26
04	Vaccine	4.93	6.70	4.04	5.13	4.45	5.21	6.71	9.95	8.24	3.26
05	Vaccine	2.05	4.03	3.91	3.32	2.35	3.11	3.67	1.79	4.63	2.20
07	Vaccine	4.14	3.88	5.06	4.79	5.12	4.15	6.19	6.65	7.24	2.92
10	Vaccine	16.44	18.10	16.28	16.54	12.76	10.20	29.99	17.40	24.23	3.03
14	Vaccine	24.68	53.85	74.21	40.07	26.14	55.02	91.51	25.03	59.30	3.13

Conclusions:

- Considerable inter-laboratory variation in antibody titres was observed (**Table 1**).
- Use of a standardized HPV16 antibody sample markedly improved the inter-laboratory assay comparability (**Table 2**).
- Establishment of an International Standard for HPV16 antibodies would facilitate comparison of HPV antibody measurements between laboratories and assays (use of an International Unit [IU]).

3.2 The first WHO international collaborative study on the standardization of HPV DNA detection^{1, 3}

Objectives:

- Assess the performance of HPV DNA detection assays in use by **29 laboratories** worldwide.
- Determine participating laboratories':
 - Correct identification of HPV types in a background of human DNA,
 - Identification of HPV types in presence/absence of other HPV types, and
 - Analytical sensitivity of detecting HPV16 and HPV18.
- Examine the feasibility of generating International Standard HPV DNA reagents (recombinant plasmids with full-length HPV-cloned genomes, in a background of HPV-negative cervical cells).

Methods:

- A panel of 24 HPV DNA clones were prepared in serial dilutions for inter-laboratory analysis including HPV types 6, 16, 18, 31, 33, 35, 45, and 52 in various combinations/dilutions.

Results:

- The majority of laboratories accurately detected HPV types at high concentrations.
- HPV16 and 18 detection were not compromised by the co-detection of other high risk [HR] genotypes.

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	HPV type data sets							
	16 ^a	18 ^a	6 ^b	31 ^b	33 ^b	35 ^b	45 ^b	52 ^b
Proficiency results (%)	63	74	71	43	95	71	95	71
Rate of false-positives ^c (%)	21	26	24	0	0	0	0	9.5
Rate of false-negatives ^d (%)	25	0	5	57	5	29	5	19
Total number of data sets (n)	24	23	21	21	21	21	21	21

^a Detection of HPV types 16 or 18 at a dilution of 10⁶ genome equivalents per milliliter, no false positive results, and no detection out of logical order.
^b Detection of HPV types 6, 31, 33, 35, 45 and 52 at a dilution of 10⁶ genome equivalents per milliliter and no false-positive results.
^c Determination of false-positive percentage is calculated by dividing the number of data sets with false positives by total number of data sets.
^d False negatives are data sets with negativity at 10⁶ genome equivalents per milliliter.

Conclusions:

- Use of recombinant HPV DNA constructs to develop International Standards was verified. Primary focus is now developing standard reagents for HPV16/18 detection, followed by next most prevalent HR types.
- Use of HPV DNA standards will vary depending on the setting and would require evaluation:
 - Clinical vaccine trials: require highly sensitive HPV DNA detection assays,
 - Management of genital HPV-related clinical disease: require less sensitive assays, and
 - High prevalence of HPV DNA in genital infections v clinical disease has demonstrated that overly sensitive HPV detection = excessive triage of women for diagnosis / treatment.

Editorial notes

- The mutual goal of the International Collaborative Studies was to provide **consistent** and **comparable** results in research and clinical settings for disease prevention and control.
- Considerable inter-laboratory variation was observed in both estimated HPV antibody levels as well as in HPV type-specific DNA detection. Consequently, the major outcome was an underlying necessity for:
 - Standard operating procedures,
 - Quality control panels, and
 - Reference reagents.
- These International Standards (Reference reagents / control panels) should fulfil the following criteria:
 - Consistent performance,
 - Long-term stability (under selected storage conditions),
 - Readily available and renewable, and
 - Mimic properties of actual biological samples under measurement.
- More details at http://www.who.int/biologicals/reference_preparations/establishment/en/index.html

4. RECENT/ UPCOMING MEETINGS

- **WHO workshop and practical course on HPV genotyping and HPV16/18 serology**

June 4th – 8th, 2007,

Institut de Microbiology, CHUV, Lausanne, Switzerland

- This was the first meeting of the Global HPV LabNet, and was organized as a joint workshop and practical course.
- The aims were to review and promote progress of the HPV LabNet and provide a practical and theoretical training to personnel from the regional reference laboratories.
- 16 members from the 6 appointed HPV LabNet laboratories (including organizers, heads / delegates and staff participated, in addition to representatives from WHO and NIBSC.
- The practical course included two major foci of laboratory assays:
 - An in-house DNA genotyping technique using patient cervical cytology samples, plus the Global HPV LabNet DNA proficiency panel,
 - Pseudovirion / virus-like particle-based ELISAs and neutralization assays for HPV16 and 18 antibodies using patient sera as well as the candidate international standard sample for HPV16 antibodies.
- Specific tasks of the HPV LabNet were set out which included:
 - Writing of the WHO Laboratory Manual for HPV diagnosis (which will contain a chapter on the role of the HPV LabNet and importance of HPV surveillance as part of HPV vaccination programs). The manual will also contain an example of the Standard Operation Procedures (SOPs) on methods that work. A WHO consultation with external experts examining each section of the manual will be held in January, 2008,
 - Design of the HPV LabNet communication strategy including a website and a bi-annual Newsletter, of which this forms the first.
- A comprehensive meeting report will be made available online at the WHO website.



LabNet Group photo from WHO workshop, June 2007, Switzerland

- **WHO consultation of the Human Papillomavirus Expert Advisory Group (HEAG)**

September 3rd – 5th, 2007,

WHO Headquarters, Geneva

- Professors S. Garland and D. Nardelli-Haeffliger represented the HPV LabNet at this meeting.
- The HEAG collated and reviewed current evidence needed to inform future discussions relating to:
 - Level of evidence needed to support possible recommendations on HPV vaccine use by the Strategic Advisory Group of Experts (SAGE), WHO's leading advisory body on immunization matters,
 - High priority research relevant to HPV vaccine introduction,
 - Increasing evidence-based decision-making about HPV vaccine introduction and the safe use of HPV vaccines, and
 - Advice to assist WHO, and its Member States, about reducing the incidence of HPV vaccine preventable diseases, including cervical cancer
- Recommendations of HEAG about prevention of HPV-related disease and possible future SAGE recommendations about HPV vaccines are intended to guide decision-makers in countries which are considering HPV vaccine introduction as a complement to other available cervical cancer prevention and control strategies such as health education, screening, and treatment. For countries considering vaccine introduction, these groups also provide technical advice on methods to ensure that vaccines are safe, reliable, and effective, delivery methods, and monitoring and evaluation of vaccination programmes, as well as other topics.

- **Informal HPV LabNet meeting to enhance the progress of the network**

November 9th, 2007, Beijing, China

At the forthcoming, 24th International Papillomavirus Conference and Clinical Workshop: to be organized by Prof J Dillner, on behalf of the WHO HPV LabNet.

5. PROJECTS ONGOING

- Development of International Standard for anti-HPV type 16 antibody – *Report to be reviewed by WHO Expert Committee on Biological Standardization (ECBS), October 2007,*
- WHO Collaborative Study for Development of International Standards for HPV 16 and 18 DNA - *Data to be reviewed by November 2007,*
- Development of Global HPV Laboratory Manual – *1st draft is under development*
- Preparation of a Proficiency Panel for HPV DNA Detection – *In process.*

More activities are in the pipeline...

6. REFERENCES

- ¹ Pagliusi SR, Dillner J, Pawlita M, Quint WG, Wheeler CM, Ferguson M. 2006. Chapter 23: International Standard reagents for harmonization of HPV serology and DNA assays – an update. Vaccine 24S3: S3/193-S3/200.
- ² Ferguson M, Heath A, Johnes S, Pagliusi S, Dillner J; Collaborative Study Participants. 2006. Results of the first WHO international collaborative study on the standardization of the detection of antibodies to human papillomaviruses. Int J Cancer 118:1508-1514.
- ³ Quint WG, Pagliusi SR, Lelie N, de Villiers EM, Wheeler CM; World Health Organization Human Papillomavirus DNA International Collaborative Study Group. 2006. Results of the first World Health Organization international collaborative study of detection of human papillomavirus DNA. J Clin Microbiol 44:571-579.

7. USEFUL WEB LINKS

http://www.who.int/vaccine_research/diseases/hpv/labnet_call/en/index.html

www.who.int/hpvcentre/en

<http://www.who.int/immunization/en/>

<http://www.who.int/biologicals/en/>

http://www.who.int/vaccine_research/diseases/hpv/TORHPVlabnetfeb06.pdf

- **Call for contributions for the 2nd WHO HPV LabNet Newsletter:** please forward suggested contributions within the next four months to suzanne.garland@rch.org.au. For example, local initiatives, pertinent projects, prevalence data for HPV DNA especially genotype specific ,sero surveys would be welcome.

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