HPV LABNET MEETING

2024-February-23

Attendees:

27 participants from 11 NRL. Norway sends apologies and gave their updates to be presented.

Alejandra Picconi	Argentina
María Dolores Fellner	Argentina
Mariel Correa	Argentina
Gerald Murray	Australia
Rita Pereira	Belgium
Michael Peeters	Belgium
Elizaveta Padalko	Belgium
Jean-Luc Prétet	France
Steffi Silling	Germany
Iwao Kukimoto	Japan
Oštrbenk Valenčak	Slovenia
Mario Poljak	Slovenia
Sara Arroyo Muhr	Sweden
Joakim Dillner	Sweden
Emel Yilmaz	Sweden
Camilla Lagheden	Sweden
Carina Eklund	Sweden
Şimal Özbek	Turkey
Yalın Kılıç	Turkey
Murat Gultekin	Turkey
Linzi.Connor	UK
janathan.danial	UK
Ira Rajbhandari	USA
Juanita Onyekwuluje	USA
Nannan Jiang	USA
Sonya Pantel	USA
Troy Querec	USA

MEETING NOTES:

1. **Introduction (Joakim Dillner, IHRC, 5 min)**

   Joakim welcomed the participants and thanked them for willingness to collaborate that has since the last meeting resulted in major, joint progresses – to be reviewed at this meeting.

2. **Preliminary results for the Screening + Genotyping proficiency studies 2023 (Carina Eklund, 13 min+3 min discussion)**

   The reports with the results from the proficiency panels for 2023 will be sent next week to all participants. A technical report will be written and uploaded at the Ref Center website:

   https://www.hpvcenter.se/publications/reports/

   In summary, HPV proficiency keeps increasing. The genotyping panel achieved 79.5% proficiency in 2023, and the screening panel looks very similar.

   - HPV 16 at 100 copies: We have in 2023 included HPV 16 and HPV 18 samples with 100 copies/ul as the assay Hybrid Capture (validated and approved by FDA) was commonly used in Latin America, and the Hybrid Capture did not detect HPV 16/18 at 10 copies/ul, translating into being classified as non-proficient. However, this year, only one laboratory still used Hybrid Capture. We will therefore not include the concentration of 100 copies/ul any more.
   - Separating HPV 35 from the pool. HPV 35 was by WHO proposed to be upgraded to a medium oncogenicity virus (previously was a low oncogenicity virus included in the panel as a pool with other low oncogenicity types. HPV 35 is prevalent in subsaharian Africa. Therefore this HPV type will in the 2024 panel be added individually as well.

3. **Preliminary results from the international viral load study (Emel Yilmaz, IHRC, 13 min+3 min discussion)**

   Preliminary results included data from Australia, Belgium, Germany, Italy, Scotland, Slovenia, Sweden and USA (Awaiting for results from France and Turkey). Most of the genotypes showed higher amounts of virusconcentration in cases than controls, except for HPV 18 and 56. HPV types ranked by oncogenicity (if a sample was positive for both HPV 16 and 31, then its was classified as HPV 16 positive (the type most oncogenic)) gave similar results as compared to ranking by which HPV type present had the highest viral load.

   The preliminary data will be presented by PhD student Emel Yilmaz, at Eurogin.

4. **Status of the HPV laboratory manual and e-learning resources (Sara Arroyo, IHRC, 13 min+3 min discussion)**

a) HPV laboratory manual: There has been substantial progress in the HPV laboratory manual. 3 chapters are finished, CDC is to review 3 chapters and write 1 chapter. Belgium to review 1 chapter. We need efforts to write: HPV serology – ELISA, HPV International standards and Setting up an HPV laboratory.

Chapter	Write	Review
1. Introduction	Sweden	CDC
2. HPV taxonomy	Sweden	CDC
3. Laboratory quality assurance	Turkey/Argentina/UK	CDC/Sweden
4. Collection and handling of specimens for HPV testing.	CDC	France, Australia
5. Nucleic Acid extraction	France/CDC/Norway	Germany
6. HPV detection and typing	Slovenia/Belgium/Norway/Sweden	CDC/France
7. HPV serology – ELISA	Pinto	Scotland/CDC
8. HPV International standards	NIBSC	CDC/Sweden
9. Data management	Kate/Sweden	Belgium
10. International and National HPV Reference Centers	Sweden	CDC
11. Setting up an HPV laboratory	Julia Gates/Pharta Baso to suggest someone	Argentina/UK

- We decided that for the red chapters (7,8 and 11), we will switch the reviewers as writers, and writers as reviewers.

b) E-learning courses

We need to provide e-learning resources with the essential parts of the chapters in the Manual. We have the chapter 2 of the manual recorded as an online course, which can be used as an example.

All chapters should be also presented as an online course. It is particularly advantageous for PhD students and postdocs (as preparing an e-course is an educational merit). If anyone is unsure of how to it technically, IHRC can help you with it.

5. **Updates from each NRL (3 minutes each, no slides)**

ARGENTINA

Supporting vaccination:

- Generation of information on the locally circulating HPV genotypes: a new study focusing on the impact of HPV vaccination in young women is ongoing (preliminary results indicate a marked drop of the vaccine oncogenic genotypes plus a drop in HPV 6 and 11). As a few vaccinated HR-genotypes were detected in vaccinated women, their whole genome is going to be analysed by NGS in collaboration with the International Reference Center.
- Since January 2024, the national HPV vaccination scheme has changed to a single dose and an active surveillance of HPV genotypes circulation has started to be implemented, which is going to be led by the HPV NRL.

Supporting HPV screening

- In collaboration with the International Reference Center, a regional HPV DNA Screening Proficiency Study was organized, through the distribution of the Global 2022 Screening panel, which allowed the assessment of 19 labs. In the same way, during 2023, a new regional Screening Proficiency Study was organized, in which labs from Argentina and countries from LA and the Caribbean (Belice, Antigua & Barbuda and Paraguay) have participated.
- A new regional screening proficiency study was carried out, with the participation of 30 Labs from the public and private health system.

Other ongoing local and regional activities

- Training and updating, advice and assistance to countries from LA and the Caribbean
- Participation in updating the WHO document regarding HPV- TPP for new HPV screening tests
- Ongoing Local studies: HPV genotyping by PCR+RLB and/or NGS in H&N cancers and in paired cervical and plasma samples and to address HPV Triage: HPV DNA Detection in plasma samples and detection of E6 / E7 oncoproteins in cervical samples

AUSTRALIA

Main work towards:

- Genotype surveillance to look at vaccination impact.
- Systematic re-review of HPV negative cancers
- Vaccine effectiveness and genotype prevalence (Vietnam, Ethiopia, South Africa)

BELGIUM

<u>HPV Surveillance</u>

- What :
  - 1200 ThinPrep collected yearly
    - Women < 35 years from the three regions of the country
    - Samples from centers taken for screening purposes
      - Infection status and vaccination status unknown
- How :
  - Test for HPV
  - If positive → Genotyping
- Why :
  - Burden of HPV in Belgium
  - Impact of vaccination on the prevalence of HPV genotypes
- Legal issue with linkage to vaccination database that must be solved

<u>Proficiency test</u>

- When :
  - 1st year = 2024 (before : 3x pilot studies)
- What :
  - Samples prepared by mixing ThinPrep
    - Some educational samples (low viral load)
- How :
  - Repeatability, reproducibility, robustness, uniformity, homogeneity, stability
  - Panel sent to ~ 40 Belgian diagnostic laboratories
  - General and individual reporting sent to participating laboratories
- Why :
  - Evaluation of laboratories to detect hrHPV
  - Reporting of genotyping not mandatory but for educational purposes

<u>Advice on testing strategy</u>

- ISO 15189 accreditation mandatory for HPV testing
- Free of choosing the assay
- Marc Arbyn
  - List of recommended molecular assays
    - For detecting hrHPV in primary screening
    - Validated according to international criteria (Meyer + VALGENT)
    - May have an impact on test reimbursement for the patient

FRANCE

- Study of HPV distribution in 400 HIV-negative MSM at the anal and pharyngeal levels using self-sampling. Prétet et al. J Med Virol 2023
- Ongoing study to determine the prevalence and distribution of HPV in the cervix and penis using self-sampling in the general population of 18-25 year old females and males. This study will allow to describe for the first time the HPV distribution in the penis. In addition, the efficacy of HPV vaccination in women will be calculated.
- HPV and environment: A feasibility study for the detection of HPV was performed on 10 wastewater samples from France and French Guyana. The results are very encouraging as HPV was detected in 8/10 samples.
- We are working towards accreditation (ISO15189) of ddPCR for HPV16/18 genotyping and quantification. Since there is no organized external quality assessment, we will propose an interlaboratory comparison. Samples will be prepared and sent to participating laboratories. All members of the HPV LabNet are welcome to participate. Please do not hesitate to contact us.

GERMANY

In Germany the NRC are re-evaluated every three years (we are in year 2 of the current period).

In 2023 we were engaged further in the tasks of the major institution responsible for the quality assurance of laboratory testing in Germany. Here, we provide HPV-positive material for the creation of the proficiency testing samples, we are reference lab for the pre-testing, responsible for the setup of the test panel, and the evaluation of the reported laboratory results.

One of our research foci is on anal cancer, currently we are performing biomarker studies to improve screening strategies (talk at EUROGIN in Stockholm).

In January 2023, we joined a collaboration of the Technical University Munich (TUM) and their partners in Africa. The aim of this initiative is addressing different research questions with respect to cervical cancer screening, but also in the end, to put up a cervical cancer screening program in Ghana. We were contacted to contribute with our expertise on HPV-testing. In February I travelled to Ghana to meet the collaboration partners and to get an impression of the local conditions concerning, health services, laboratories (and equipment), and so on.

I used the opportunity to transfer two of the LabNet HPV-screening panels to two labs in Accra. Also, a colleague of mine tried to send three additional sets to labs in Addis Ababa (Ethiopia), Ifakara (Tanzania) and Yaoundé (Cameroon).

Finally, I would like to find out if and how the European Union-based NRC are affected by the “in-vitro diagnostics regulation”.

JAPAN

Japan has a low vaccination rate (30%). Two-doses, 9-valent vaccination has just begun last year in Japan.

The declining trend in HPV16/18 prevalence among young women with ICC is already visible. The vaccination rate for that age group was about 70%.

Preliminary results are published here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10637081/

NORWAY

- *Organisational changes:*
  Kristiane taking over as head of the Norwegian reference lab as of February 1st 2024. Irene continues in a 20 % advisory position. In August we will advertise a new position.

- *Quality assurance project on HPV-negative cervical cancers:*
  In 2024, we will start a quality assurance project on HPV-negative cervical cancers in close collaboration with the Cancer Registry of Norway. We will perform extended genotyping and E6E7-directed PCR on all HPV-negative cancers dating back to 2015. The project will primarily last for 5 years. Any false HPV-negative samples showing results of interest for whole genome sequencing will be included in a separate research project to identify possible genomic rearrangements, deletion or mutations causing the initial HPV-negativity.

- *Research:*
  Work using TaME-seq for HPV-whole genome sequencing is ongoing, with one postdoc finishing in February and one PhD-student soon submitting the thesis. TaME-seq is used to identify chromosomal integration sites, deletions and mutations in the HPV-genome, as well as investigating HPV intra-host variability and identification of low frequency variants. By using TaME-seq, we aim to identify new biomarkers in HPV-positive women. This work will continue and we plan to apply for external funding in 2024.

  We also plan to establish a new research biobank representing the current screening population and are currently in the process of getting the necessary approvals.

- *Self-sampling in the national screening program:*
  Is currently being implemented through a three-year period. Copan Floqswab in combination with BD Onclarity are used for analysis. The reference laboratory is involved in quality assurance of the procedures.

- *HPV-vaccine surveillance:*
  Ongoing work to monitor vaccine effectiveness, adverse effects and vaccination coverage, administered by the Norwegian Institute of Public Health. The Norwegian NRL is managing HPV-

analysis of tissue samples from high-grade lesions and cancers, to study changes of HPV genotype distribution over time. Data will be presented at Eurogin.

SCOTLAND

New assay

SHPVRL recently adopted Allplex HPV28 as a replacement for Anyplex II HPV28 and is currently going through the process for extension to accreditation scope.

HPV Surveillance

We recently completed retrospective testing of samples obtained from MSM. A complete dataset was sent to Public Health Scotland, who noted an increase in HPV11 in 2019 compared to other years. Analysis is still ongoing.

Surveillance starting once again on LBC samples taken at cervical screening appointments from 2024-2030. Aiming to assess the duration of protection in both bivalent (from 2024) and quadrivalent (from 2026) vaccines, while investigating any change in herd protection against HPV31/33/45 following the introduction of the quadrivalent vaccine.

Projects

SHPVRL are looking to assess acceptability and feasibility of HPV vaccine uptake in vulnerable women as well as investigating HPV type prevalence in recruited women. Vulnerable women include those who are immunocompromised or immunosuppressed, suffer from homelessness, are involved in transactional sex and/or drug use, or are in the justice system. Another project, in collaboration with DAYE, is a diagnostic trial comparing diagnostic accuracy of a tampon with self-collected and clinician-taken vaginal swabs. SHPVRL are also progressing through the steps to obtain funding for NGS of HPV-negative cervical cancers (negative by PCR in the first instance).

Others:

There was an article published in HPV World reporting on challenges and considerations for HPV self-sampling.
The self-sampling journey: technical considerations and challenges (hpvworld.com)

There is also an article on resuspension volume in self-samples published last year:
https://doi.org/10.2144/btn-2022-0084

We were also involved in an interlaboratory comparison study with Slovenia (University of Ljubljana), testing LBC samples from cases and controls on Allplex HR as part of a validation to fulfil the Meijer criteria. https://doi.org/10.1016/j.jcv.2023.105638

SLOVENIA

Main work towards:

- Following the HPV test commercial market. 5th global inventory (At least 264 HPV tests + 511 variants)
- Research studies for HPV prevalence (VALGENT) → 3500 thinprep samples and 1200 blood samples to assess HPV prevalence. Collaborations are welcome.

TURKEY:

Turkey was the first country to implement HPV based screening in the world. The program works nationwide since 2014. Until 2023, Ministry of Health tender asked for FDA approval or validation by Meijer Criteria. However, the latest tender did not ask for analytical or clinical accuracy or validation data, but rather only asked for capacity to detect and genotype HPV 16, 18 and 45 individually vs. others. The national laboratory is also being transferred to work under the guidance of Department of Microbiology Reference Laboratories rather than Cancer Control Department. Additionally, pap-smear check for positive cases was not asked during the tender, so patients are only screened by HPV DNA PCR kit. Mrs. Şimal now works in the new company and works in close collaboration with me and Dr. Yalın for further validation studies of the new kit and results for accuracy, concordance that will be released very soon by the team. The company is open for close collaboration and Sımal, me and Yalın will help on further scientific research.

On the other hand, the other Turkish kit developed in collaboration with Karolinska, Hacettepe University (Murat Gultekin) and Izmir Economy University (Yalın and Simal) has now been started to be piloted in Ghana and Democratic Congo. The results will be evaluated and published soon,

Turkish Viral load study has just recently started. Due to the new position of Sımal in the Turkish reference laboratory, the study has been delayed but the team will finalize the study as soon as possible

SOUTH AFRICA (non-attendence)

SWEDEN

The National HPV Reference Laboratory of Sweden focuses on confirmatory testing where every year we see an increase in specimens sent to us. During 2023 we received 68 samples. HPV positivity in apparently negative HPV HSIL+ is in agreement with what the LabNet just published.

Furthermore, we assist other laboratories when issues with HPV testing arise, both with sending plasmids for validation and quality assurance or by analyzing their samples at our laboratory.

USA

- Working to complete the chapter/review for Mid march

- Method development, HPV enriched whole genome sequencing assay, nanostring platform for HPV detection.
- Clinical surveillance (completed the data for nineplex elisa analysis for serum. Abstract will be presented at Eurogin.
- Typeseq (ngs) monitoring of HPV types in precancers and cancers,
- Respiratory papillomatosis
- Anal HPV among MSM.
- Collaboration with Botswana.
- Globe HPV study with international vaccination institute.

6. Future of LabNet and final discussion (Joakim Dillner, IHRC, 45 min)

A) It is now widely recognized that we stand for quality and reproducibility in this field. We need to continue with the proficiency panels and exchanging experiences. How can we build a sustainable business model?

What can we do to support laboratories to continue being reference centers?

Many NRL are re-evaluated after some years. The appointment of the government is temporary. It would be helpful to know how we can support them to fulfil the demand of their evaluations.

What can we do is also to promote that more countries should appoint national reference laboratories.

Argentina is already discussing with Colombia and Mexico the possibility of having an NRL from these countries.

B) There is a scaring increase of HPV tests that are being used but are not approved, validated, or even evaluated. From the LabNet, we could:

- Publish a guidance from the LabNet about the tests to be used, and not to be used. 90% is in private area, no reimbursement, making business and promoting overscreening.
- Scientific Paper about LabNet. There is already a chapter about the LabNet (Chapter 10) and our Laboratory Manua. We should describe clearly the requirements and responsibilities of the NRLs and highlight the importance of quality.
- Evaluation and validation of tests: It is estimated that 1,5 to 1.8 billion of HPV tests will be needed for cervical cancer elimination (if each woman is screened twice, at 35 and 45 years). We need to help out with validation of the inexpensive tests that will be required. We have the samples, the knowledge and the credibility for this.

Thank you for participating! We see you at Eurogin!