HPV LABNET MEETING

2024-October-18

Attendees:

25 participants from 11 NRL.

A Technical problem had resulted in that both Australia and Japan had not received the invitation.

Alejandra Picconi	Argentina
María Dolores Fellner	Argentina
Mariel Correa	Argentina
Rita Pereira	Belgium
Elizaveta Padalko	Belgium
Sharon Dhillon	Belgium
Jean-Luc Prétet	France
Steffi Silling	Germany
Ulrike Wieland	Germany
Kristiane Söreng	Norway
George Obregon	Peru
Lesly Solis	Peru
Susan Espetia	Peru
Maribel Acuna Barrios	Peru
Noel Gahamanyi,	Rwanda
Mario Poljak	Slovenia
Sara Arroyo Muhr	Sweden
Joakim Dillner	Sweden
Emel Yilmaz	Sweden
Camilla Lagheden	Sweden
Carina Eklund	Sweden
Kate Cuschieri	Scotland

Juanita Onyekwuluje	USA
Gitika Panicker	USA
Nannan Jiang	USA
Troy Querec	USA

MEETING NOTES:

1. **Introduction (Joakim Dillner, IHRC, 10 min)**

Joakim welcomed the participants, particularly the 2 new members and thanked them for willingness to collaborate.

Regarding proficiency studies, technical reports and published papers are written and uploaded at the Ref Center website: https://www.hpvcenter.se/proficiency_panel/. In summary, HPV proficiency keeps increasing, reaching 79% for the genotyping panels and 91% for the screening panels in 2023.

Results from the 2022, 2023 genotyping panels were published in the October issue of J Med Virol (https://pubmed.ncbi.nlm.nih.gov/39439211/).

2. **New NRLs members (10 min)**

Perú:

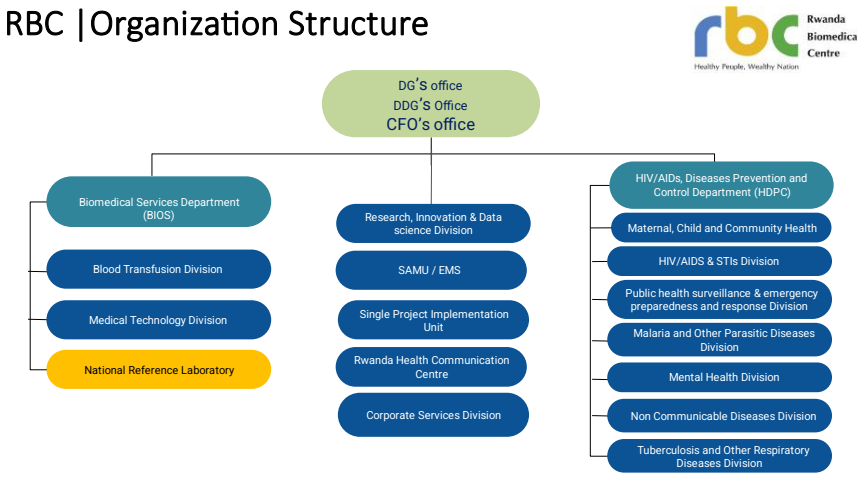
The National Reference Laboratory for Human Papillomavirus (NRL HPV) is an integral part of the National Reference Laboratory for Sexually Transmitted Viruses (NRL STV) at the National Institute of Health of Peru (NHI). The technical team is organized into four specialized areas: serology, cytometry, molecular biology (HBV, HCV and HIV viral load and PCR) and genotyping. Notably, the NRL STV collaborates closely with the National Cancer Program (PNC).

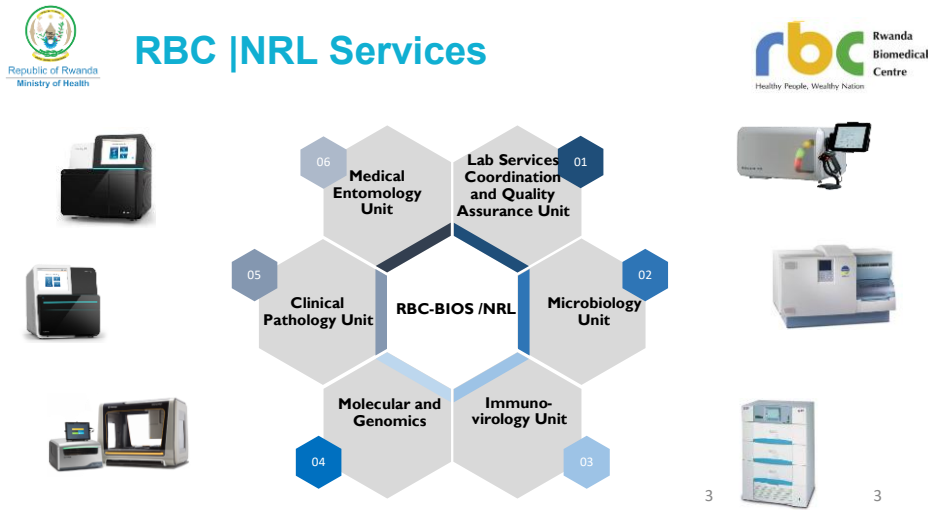
Since 2022, one significant achievement has been the decentralization of molecular HPV screening across 19 of the 29 regions in Peru. This initiative was facilitated by the implementation of the Cobas® 4800 HPV system in first-level healthcare laboratories, resulting in nearly 189,000 tests conducted in 2023. Furthermore, the NRL ensures high-quality standards through external assessments conducted by panels from the College of American Pathologists (CAP) and soon from the International Human Papillomavirus Reference Center (IHRC).

In addition to these activities, we are actively developing normative documents for cervical cancer screening and enhancing interoperability between the information systems of the Ministry of Health and NHI. Our research efforts also focus on evaluating diagnostic tests and preparing for the surveillance of circulating HPV genotypes within the Peruvian population as a method for surveillance of effectiveness of the HPV vaccination program.

Rwanda:

- Dr. Noel Gahamanyi, Ag. Division Manager of the National Reference Laboratory (NRL) at the Rwanda Biomedical Centre (RBC) in Rwanda introduced the NRL as a new member and explained to the audience where NRL fits within the RBC organigram. He described that the NRL is composed of six units including (i) Microbiology, (ii) Immunovirology, (iii) Molecular Genomics, (iv) Medical Entomology, (v) Clinical Pathology, and (vi) the Laboratory Services Coordination and Quality Assurance. HPV testing is coordinated by the Immunovirology Unit. The NRL is in charge of HIV testing and currently, the Polymerase Chain Reaction is used for HPV testing.
- He thanked the Labnet to welcome Rwanda as a new member and committed to process the PT to be received within the specified time. He said that this will increase the reliability of results provided by the NRL.
- Finally, he requested that the LabNet should go beyond routine testing and also promote international quality of sequencing as Rwanda is also running sequencing of HPV.





3. **Status of the HPV laboratory manual and e-learning resources (10 min + 5 min discussion)**

- Chapters 7 and 8 are under final revision status and Chapter 11 will be written and incorporated into the manual after publication.
- We will publish it at hpvcenter.se and as it is an interactive manual, all edits, updates, etc, are welcome and will be incorporated.
- Once finished, we will seek endorsement from Maribel Almonte (WHO).
- Maribel Almonte will also be asked about writing a new chapter based on the WHO Technical Product Profile (TPP) for HPV (which are to be released in a month)
- We will write a short summary of the manual in a medline-indexed journal (All authors and reviewers will be coauthors).
- 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. The other chapters are also suitable as basis for an online course.

Chapter	Write	Review
1. Introduction	Sweden	CDC
2. HPV types	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	Belgium/Norway/Sweden	France and Slovenia
7. HPV serology – ELISA	Pinto, USA	Sweden
8. HPV International standards	Dianna Wilkinson, UK	Sweden
9. Data management	Kate/Sweden	Belgium
10. International and National HPV Reference Centers	Sweden	CDC
11. Setting up an HPV laboratory	Argentina/UK/Germany	Julia Gates/Partha Basu to suggest someon

4. **International study on virus types and amounts in CIN2+ (10 min)**

A collaborative study including 10 national reference laboratories (NRLs). For study, 101 cases and 202 controls were collected in Sweden. The cases were a consecutive series of histopathology-verified CIN2+ identified among women resident in the capital region of Sweden which had had a cervical liquid-based cytology sample taken at most three months before the histopathological diagnosis. Controls were identified among women resident in the capital region of Sweden who were participating in the population-based cervical screening program. Only primary screening samples were eligible (not reflex samples or samples from disorganized screening). Two controls taken at the same time as the matched case and matched by ± 5 years of age were identified.

Aliquots from all samples were sent with standards for calibration to national reference laboratories. Result considered to be in consensus if there was >70% agreement.

Cohen's Kappa for HPV types ranged between 0.76-0.92. However, to be able to accurately determine type specific virus amounts in samples, a new set of standards for calibration (1, 10, 100, 1000, 10000, 100000 copies/ul for each HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) are being currently distributed to the participating laboratories.

5. **Updates from each NRL (3 minutes each)**

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
- In 2024, an active and permanent surveillance of the circulation of HPV genotypes began to be implemented, which will be led by the HPV NRL.

Supporting HPV screening

- In collaboration with the IHRC, participation in the Global HPV DNA Screening Proficiency Studies were locally organized: -
    - 2022: Participation of 19 labs from Argentina (HPV National Laboratory Network) and 1 country from LA, all of them using the HC2 test.
    - 2023: Participation of 18 labs from Argentina (HPV National or Provincial Laboratory Networks) and 4 countries from LA&C region.
- Locally inter-labs studies since 2019; in 2024: participation of 63 public and private labs.
- Thinking about developing a tool (panel of samples) that let us evaluate the performance of new HPV tests.
- Participating in the 2024 *Updates on the Recommendations of the Comprehensive Strategy for the Prevention of Cervical Cancer*, in the frame of the National Health Ministry.

Other ongoing local and regional activities, including:

- The training and updating, advice and assistance, supporting implementation and QA to countries from L&Cregion in collaboration with PAHO.
- Some ongoing Local studies: - HPV DNA detection in plasma samples to address its usefulness for HPV triage. - Planning a study to analyse the urine sample for the HPV-based screening for cervical cancer prevention and vaccine surveillance.

BELGIUM

As Belgium is switching on the 01/01/2025 to the HPV-based national cervical cancer screening programme, our NRL is currently actively involved in the preparatory work necessary for this switch. It has been decided that only the restricted number of HPV tests that are clinically validated according to the international guidelines will be allowed to be used by the clinical laboratories as an HPV-test within primary cervical cancer screening. The information regarding the tests that will be allowed to be used is already available on the NRC website and will be

updated regularly, the last update was on the 05/08/24: National Reference Center (NRC) for Human papillomavirus | sciensano.be As the NRL is in Belgium a temporary assignment based on the competitive call for 41 National Reference Centres in Medical Microbiology, there was a new call launched by the Belgian National Public Health Institute, Sciensano, in 2024 for the application for all NRL functions, including for HPV. The current NRL-consortium has applied and we have won the call and will continue to serve as NRL also for the years 2025-2029.

FRANCE

The clinical relevance of grey zone HPV results (above the analytical cutoff but below the clinical cutoff) obtained with the Alinity HR HPV was investigated. Of the 102 women with such results, none but two presented a CIN2+ lesion within the next 22 months. The two women with HSIL had a history of persistent infection. It is therefore important to limit HPV-positive results to those above the clinical cut-off (Node et al, J Med Virol 2024)

The intra- and inter-reproducibility of new hrHPV tests (Ampfire test, Sansure HPV Diagnosis Kit and Papilloplex) was evaluated between the Belgian and French NRLs. After analysis of 550 cervical specimens, 1/3 of which were previously tested hrHPV positive, all 3 tests showed excellent reproducibility and met one of the 3 Meijer criteria for use in cervical cancer screening.

A large epidemiological study focusing on HPV infection at the vaginal and penile levels was conducted in the French/ general population using self-sampling. Of the 600 vaginal and 400 penile specimens collected from participants aged 18-29 years and tested using the INNO-LiPA® HPV Genotyping Extra II test, half and one third, respectively, were HPV positive. This indicates a high prevalence of HPV in the young French population. (Prétet et al, J Med Virol, 2024 ; Chung et al, J Med Virol, 2024 ; Chung et al, Manuscript in preparation)

The feasibility of detecting and quantifying HPV DNA from wastewater was tested using samples collected in France and Guyana. HPV DNA is frequently detected, but reproducibility is low, indicating likely low HPV titers in wastewater. Specific protocols for HPV DNA enrichment and extraction are being developed to increase the robustness of the HPV DNA detection.

GERMANY

- Re-evalution of the NRL will be next year (for a 3-year period)
- Supporting International HPV standard (samples arrived today) and the viral load study
- Taking part in the HPV Proficiency Panels and organizing laboratories in Africa (namely two in Accra/Ghana) to also take part
- Currently, we are picking up other aspects of HPV-associated diseases e.g. focal epithelial hyperplasia (FEH; see IPVC abstract #2215), juvenile onset recurrent respiratory papillomatosis (JORRP)
- A multicenter AIN treatment study is about to be published (see IPVC abstract #2273)
- It is planned to take part in a nation-wide study organized by the Robert Koch-Institute on public health aspects. We will be involved in oral and genital HPV testing of >5.000 samples (HPV epidemiology and to monitor vaccination impact).

NORWAY

- *TaME-seq:*

We continue development of TaME-seq, our method for HPV whole genome sequencing. The method is used for identifying chromosomal integration sites, deletions and mutations in the HPV-genome, and for investigating HPV intrahost variability and low frequency variants.

We are currently writing a grant proposal where we will study the interaction between HPV variations (using TaME-seq) and cervicovaginal microbiome in cervical cancer development. For this project, we are planning to establish a new research biobank, and we are working to obtain the necessary ethical approvals.

We will bring a poster on TaME-seq at IPVC, please come and see us ☺

- *Project on HPV-negative cervical cancers:*

We are starting a quality assurance project on HPV-negative cervical cancers together with the Cancer Registry of Norway. In this project we will identify and re-analyse all cases of HPV-negative cervical cancers dating back to 2015. Some of the false HPV-negative cases will be included in a separate research project and subjected for TaME-seq analysis to identify any genomic rearrangements, deletions or mutations that may have caused the initial HPV-negativity.

We have finally obtained all the necessary approvals to start.

- *Self-sampling in the national screening program:*

Self-sampling is being implemented in Norway through a three-year period (starting in 2023). The reference laboratory is involved in quality assurance of the procedures.

The project started with offering self-samples to women with special needs. Next group to be included are women who have not been tested in 10 years or more. These women will get information about self-sampling during October 2024.

SCOTLAND

Scotland has now rolling funding for NGS of apparently HPV negative cervical cancers in Scotland.

HPV immunisation surveillance of women in cervical screening restarted – with a focus on those immunised by the 4 v vaccine (to compliment earlier work on women immunised by 2v vaccine)

More research work on detection of circulating DNA in the blood of high risk patients (post cancer treatment) to gain insight into prognosis and treatment success

Recent publication on self sampling in women who have defaulted from cervical screening in a health board in Scotland: High-risk HPV mRNA testing on self-samples offered to those who do not attend for organised cervical screening – real world data from the Dumfries and Galloway region in Scotland - ScienceDirect

SLOVENIA

1. Fifth periodical inventory of commercially available HPV molecular tests on global market published (Poljak et al. J Clin Virol 2024;172:105671). At least 264 distinct commercial HPV tests and 511 variants are available globally. Small net increase in total number of tests, but strong 2020-2023 market dynamic; 86 distinct HPV tests introduced and 76 withdrawn from the market between January 2020 and December 2023. Half of the distinct HPV tests are without a single peer-reviewed publication. Published evidence of analytical/clinical performance quality is lacking for 79% of HPV tests present on the global market.
2. Validation of HPV tests following Meijer's guidelines: Allplex (ThinPrep finished; SurePath ongoing); NeuMoDx (ThinPrep, SurePath and self-samples finished). Several ongoing.
3. 9 years follow-up data (four screening rounds; 4,000 women) of Slovenian population-based screening cohort published in Int J Cancer (in press). Samples collected in 2009/2010. Four clinically validated HPV assays (Hybrid Capture 2, RealTime m2000; cobas 4800 and Alinity m) showed comparable safety and better assurance against precancerous lesions than cytology, but some important differences were identified in the performance characteristics of HPV assays impacting the referral rate.
4. Oct 2023-Oct 2024: 5,300 women recruited for new population-based screening cohort (women aged 20-64 years; 5,300 ThinPrep samples + 1,810 serum samples collected at present)
5. Ongoing: collection of ThinPrep samples + serum samples from 3,000+ women aged 20-26 years (half vaccinated in age 12; half not).

SWEDEN

The National HPV Reference Laboratory of Sweden focuses on confirmatory testing where we see an increase in specimens sent to us. During 2023 we received 68 samples and in 2024 we have received 72 samples so far.

HPV positivity in apparently negative HPV HSIL+ is in agreement with what the LabNet just published. The samples we receive correspond mostly to LBC samples (since 2019, 195/263, 74%) and FFPE (68/263, 26%). The increase in number of samples is because more laboratories and regions are now implementing the guidance. It is now 10 regions in Sweden (total regions is 21) who send us samples for confirmatory testing. Since May 2024 we have included BD Cor as a first (re)testing method and we have a new extraction method (MagLead).

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

Our collaborations include 1) comparing immunologic response to altered dosing schedules and methods of administration; 2) resuming HPV testing for US National Health And Nutrition Evaluation Survey to monitor trends in the general US population; 3) surveillance of HPV in cervical cancer and precancer; 4) HPV surveillance in special populations, including those living

with HIV, men who have sex with men and children with recurrent respiratory papillomatosis; 5) Technology transfer and support to global partners in 6 Sub-Saharan Africa countries, 3 South Asia countries; and 6) developing reference reagents for laboratory quality control and new methods to study HPV transcripts and whole genome sequencing data.

6. Future of LabNet (40 min)

The presentation highlighted two major challenges in the global effort to eliminate cervical cancer. First, the implementation of HPV screening, which is critical for reducing cervical cancer to below 4 cases per 100,000. It remains slow despite clear WHO recommendations since 2014. Current screening with cytology has much higher cancer risks compared to HPV testing, but the adoption of HPV screening has been hindered by complex test evaluation guidelines. Although HPV tests have been FDA-approved for over 30 years, global utilization is limited due to slow development and validation processes. The presentation stressed the need for inexpensive, high-performance HPV tests to expedite cancer elimination efforts. Similarly, HPV vaccine development is also affected by non-standardized HPV testing methods, further delaying progress. Secondly, the presentation addressed the need for real-time evaluation of HPV vaccination programs to measure their effectiveness. Since the incubation period between HPV infection and cervical cancer can span two decades, alternative measures, such as HPV prevalence in younger, pre-screening age cohorts, are necessary to assess the impact of vaccination. The example of Sweden, where HPV screening with extended genotyping shows a dramatic decline in HPV 16/18 prevalence among birth cohorts vaccinated in school is an example of such real-time monitoring.

Global collaboration through networks like the Global HPV LabNet and the International Cancer Screening Network (ICSN) is crucial to expand HPV screening, particularly in low- and middle-income countries (LMICs). We need help expanding our LabNet. Most of the NRL have contacts with other countries. Please help to have them onboard!

The presentation emphasized the need for simple, reliable evaluation methods for new HPV tests to facilitate rapid implementation worldwide. This is part of a new BMGF grant. If we agree on a consensus amount for adequate detection (IU/ul) for the different genotypes (e.g. HPV16/18 with 10 IU/ul, HPV middle risk (other vaccin types) with 25 IU/ul and HPV low risk with 100 IU/ul), and build a simple panel for evaluation of tests using International Standards, then we can facilitate rapid implementation of the tests worldwide.