International Collaborative Study on Sensitivity and Specificity for CIN2+ of Viral load and Types of HPV

BACKGROUND:

HPV-based screening is the recommended modality to screen for cervical cancer nowadays. The performance requirements for HPV tests to be used for cervical screening have already been established in a consensus article (Meijer et al. 2015). These requirements are based on the sensitivity for detecting CIN2+ compared to histopathology, specificity in terms of 1-positivity in population-based screening samples and on interlaboratory reproducibility.

However, this definition of performance has several problems. Histopathological diagnosis is variable, causing limited comparability between different validation efforts. Furthermore, obtaining the samples from the required number of patients can be prohibitively expensive.

The sensitivity and specificity of new HPV tests are nowadays compared to the performance of the classical HPV tests that were used in randomised trials and demonstrated superior performance in preventing invasive cervical cancer (Hybrid Capture 2 and GP5+6+ PCR) (Meijer et al., 2021). However, these reference HPV tests are old and rarely used nowadays. For virtually any virological test, the definition of performance is nowadays instead defined by using assay detection limits in amounts of virus, in addition to reproducibility.

As it is straightforward to measure the amounts of virus that are present in CIN2+ as well as in control samples from population-based screening, it should be rather simple to arrive at knowledge of which sensitivity and specificity for CIN2+ that a particular detection limit for HPV corresponds to. If this is assessed in an international collaborative study, also the aspect of interlaboratory reproducibility will have been assessed.

An international definition of desirable detection limits would greatly facilitate both quality assurance and development/validation of new HPV tests.

AIMS:

- Quantification of HPV type-specific virus amounts in women with CIN2+ and in population-based control women (sensitivity and specificity).
- Assessment of interlaboratory reproducibility in assessing virus amounts in relation to CIN2+.
- To prepare an evidence base that could be used as a basis for new international guidelines on required performance of HPV tests.

METHODS AND MATERIALS

Selection Process

The study base will comprise women resident in the capital region of Sweden (Stockholm-Gotland region). The screening program mandates HPV-based screening in all ages, with reflex cytology if HPV-positive. Women with positive cytology should be referred for biopsy and histopathology. The managing clinician may decide to deviate from recommendations, if required.

The cases and controls were selected using the laboratory database of the Center for Cervical Cancer Prevention (CCCP) of the Karolinska University Hospital that contains all records on HPV tests, cytologies and histopathologies of cervical samples. CCCP is the central laboratory of the region with an annual throughput of about 130,000 HPV tests.

Cases are defined as a consecutive sample of women diagnosed with a histopathological diagnosis of CIN2+ (CIN2 or worse including adenocarcinoma in situ (AIS) and invasive cancers (ICC)). The samples for definition of sensitivity of the screening test are identified among cervical liquid-based cytology (LBC) samples that were taken/registered not more than three months before the histopathological diagnosis (but earlier or at the same date as the sample for histopathology). All cases are eligible regardless of whether they were identified by the organised program or by non-organised testing (typically women with symptoms who are tested

at the discretion of the clinician). This is because an HPV test should be able to detect disease also from samples taken at a clinical indication.

Controls were matched to cases by age and calendar time of sampling. First, all samples corresponding to women participating in the population-based screening that were registered at the same date as the cases in the laboratory data system were identified. Then each case was individually matched with 2 controls by age (± 5 years). Although all samples have been HPV tested in the screening and HPV positives will also have cytology results, HPV testing and/or cytology results are not used in the selection of controls. The controls are thus an unbiased sample of the entire study base population and not selected based on any particular HPV test. However, the testing data is collected and can be used for comparisons. As the screening program performs repeat testing of women testing HPV-positive in self-collected samples, women with prior self-collected samples are not eligible for inclusion as controls (the controls should be a random sample of the study base population and not selected by prior testing results).

All LBC samples (Thin Prep) registered from 1st April 2022 were stored unmodified at +4 C and the laboratory database continuously checked for whether any subsequent CIN2+ in histopathology had been diagnosed.

Data on sample ID, registration date, personal identification number, HPV results, including HPV type and SNOMED codes for each analysis, if available cytology and histopathology, are recorded and saved in a secure server.

Selection success:

Cases: 100 women with CIN2+ in histopathology and a prior LBC sample. There were: 89 women with HSIL, 3 women with AIS, 3 women with HSIL and AIS, 4 women with ICC and 1 woman with suspicion of ICC.

Controls: 200 women from population-based screening. HPV, cytology and histopathology results are available but were not used for selection. There are no CIN2+ cases among the controls (not by design, this was the outcome of the unbiased selection).

In addition, we intend to prepare 2 unblinded calibration samples for each of the 13 HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) classified as oncogenic or probably oncogenic by IARC (total of 26 samples). These will be defined amounts in International Units, diluted in a defined amount of human DNA corresponding to the average amount of human DNA typically found in clinical samples.

Participating laboratories/centers

The participating laboratories will have to perform HPV typing and measure the viral load of the specimens mentioned above (326 samples, including 100 cases, 200 controls and 26 unblinded calibration samples). All specimens will be delivered in ThinPrep solution.

Requirements for participation:

Desirable: A quantitative HPV test that provides the amount of HPV DNA in the sample for all 13 oncogenic or probably oncogenic HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68).

Acceptable: An HPV test with a defined detection limit for the different HPV types. If the detection limit is given in genome equivalents rather than IU, that is acceptable. If the HPV test tests only for a more limited number of HPV types, that is also acceptable. However, if the amounts of LBC sample available is limiting, priority will be given to laboratories intending to use desirable HPV tests.

RNA: As the samples are LBC samples in ThinPrep, stable RNA should also be present. We have no quantification standards for RNA, but laboratories wishing to participate with an RNA test should contact us for a discussion.

Contact us at: hpvcenter@ki.se

Application for participating in

International Collaborative Study on Sensitivity and Specificity for CIN2+ of

Viral load and Types of HPV

Lab ID/Name:	
Department/Laboratory	
Address	
Delivery address:	
City	**Postal code**
Province/State	**Country**
E-mail	**Fax**
Phone	

Principal Investigator:
First Name
Surname (Title)
HPV DNA typing experience in your laboratory
Methodology used (may be more than one)
Annual number of HPV typing tests performed
Brief description of involvement in HPV surveillance or HPV vaccine development

Return registration form by email to: hpvcenter@ki.se