Cervical cancer is the fourth most common cancer in women globally. Infection of cervical epithelium with a human papillomavirus (HPV) can lead to the development of cervical intraepithelial neoplasia (CIN) and ultimately cervical cancer. This discovery has facilitated the development of various HPV tests (with over 125 assays commercially available to date) and two licensed prophylactic HPV vaccines.

HPV tests used in epidemiologic studies require a high analytical sensitivity and specificity and the capacity to individually identify a range of HPVs (genotyping). These tests can be used to 1) determine HPV vaccine efficacy against several surrogate endpoints for cervical cancer, from persistent HPV infection to high-grade CIN lesions (CIN2+) associated with vaccine-targeted HPVs, 2) monitor the effects of nation-wide vaccination on prevalence of HPV genotypes (and possibly type replacement) within an immunized cohort, and 3) investigate the prevalence of individual HPVs in (pre)malignant diseases.

In a clinical setting, HPV tests should detect HPV infections mainly associated with clinically meaningful disease, i.e., CIN2+ lesions (clinical sensitivity), while limiting the detection of transient HPV infections not associated with CIN2+ (clinical specificity). These assays usually target 13-14 so-called high-risk (hr)HPVs, i.e., HPVs that have the highest association with cervical cancer, in aggregate (pooled detection). This intensive use of hrHPV testing in clinical practice could be 1) primary cervical cancer screening, 2) triage of women with equivocal cytology, and 3) test-of-cure after treatment for high-grade CIN. The utility of hrHPV genotyping in clinical practice has not been established, but might be useful for HPV16 and HPV18, the types that confer the highest risk for cervical cancer.

In this thesis we have evaluated a number of established and novel PCR-based technologies for the identification of HPVs, and applied these in epidemiological and clinical studies in accordance with their intended use. The thesis comprises three parts: 1) the assessment of analytical accuracy of several recently developed assays for HPV genotyping (Chapters 2-5), 2) the application of (novel) techniques for HPV characterization in several avenues of research (Chapters 6-9), and 3) the utility of novel methods for hrHPV detection and self-sample collection within a clinical setting (Chapters 10-11).

Part 1: Analytical accuracy of HPV genotyping assays

In Chapter 2, we investigated if the novel SPF10 PCR-based INNO-LiPA HPV Genotyping Extra assay (INNO-LiPA) has sufficient analytical accuracy to be used for epidemiologic HPV research. The pooled detection of HPVs (in general) and identification of individual HPV genotypes by INNO-LiPA was compared to the original SPF 10 LiPA 25 (version 1), a globally established and analytically sensitive and specific HPV test algorithm. For this purpose, we used a selected panel of cervical swabs and biopsies, the two types of specimens mostly used in epidemiologic HPV research and HPV vaccine efficacy studies. INNO-LiPA is less suitable for these purposes than SPF10 LiPA35 (version 1), based on a decreased detection of HPVs in general (pooled detection) and of HPV types individually (genotyping) in both specimen types.

Within a clinical setting, accurate identification of individual hrHPVs might be valuable for further risk stratification of women testing positive by hrHPV-based cervical cancer screening. In Chapters 3 and 4, we present two tests with full HPV genotyping capability using amplification products generated by the GP5+/6+ PCR, a hrHPV test that has been clinically validated for cervical cancer screening in multiple, longitudinal trials. The GP5+/6+ strip (previously marketed as digene HPV Genotyping RH Test) is easy to use and suitable for low-throughput purposes, while the Luminex bead-based GP5+/6+
LMNX (previously marketed as digene HPV Genotyping LQ Test) can be applied in high-throughput settings. The GP5+/6+ strip and GP5+/6+ LMNX demonstrated high concordance with the Reverse Line Blot, an established in-house method for GP5+/6+ PCR-based genotyping, in Chapter 3 and Chapter 4, respectively.

The Amplicor® HPV test (Amplicor) is a test for pooled detection of hrHPVs and requires performance of a separate assay for the subsequent identification of HPV genotypes. In Chapter 5 we demonstrated that the amplification products generated by Amplicor can be directly genotyped using GP5+/6+ strip and GP5+/6+ LMNX. Thus, these assays offer universal genotyping capacity due to their compatibility with amplification products generated by different primer sets, e.g., GP5+/6+ and Amplicor.

Part 2: Application of HPV genotyping assays in epidemiologic studies

In Chapter 6, the SPF10 LiPA 25 (version 1) algorithm was applied to determine the prevalence of and determinants for HPV genotype infections in a selected multi-ethnic cohort prior to the start of a vaccination program in Paramaribo, Suriname. Pre-vaccination prevalence of hrHPVs was high and comparable to Latin American and other Caribbean regions. Independent determinants of HPV infection were the number of recent sexual partners, concurrent infection with Chlamydia trachomatis, ethnic sexual mixing and ethnic group. These baseline data allow post-vaccination surveillance of changes in HPV genotype prevalence within the population of Paramaribo, Suriname.

Chapter 7 describes the HPV genotype distribution in the largest worldwide collection of invasive cervical cancer biopsies (n=10,575) to date, using a common protocol for sample selection, pathology review and HPV test algorithm (by SPF10 LiPA 25 [version 1]). Despite geographical differences, HPV types 16, 18, 31, 33, 35, 45, 52, and 58 had the highest prevalence in cervical cancer and should be given priority in assessing the (cross-)protective effects of licensed and novel prophylactic HPV vaccines. Cervical cancers related to HPV types 16, 18, or 45 were diagnosed at a younger mean age than those with other HPV types, underlining their importance as targets in hrHPV-based cervical cancer screening.

In the same collection of cervical cancers, we determined the single presence of HPV types rarely targeted by current HPV genotyping tests, using a novel sequence methodology (Chapter 8). The prevalence in cervical cancer of rare HPV types classified as possibly high-risk (WHO IARC Class 2B; 2.3%), was very low compared to that of established hrHPVs (Class 1/2A; 97.5%) and therefore of minor clinical importance. However, their occurrence as single infections in cervical cancers strengthens the circumstantial evidence of a carcinogenic role, similar to the phylogenetically related Class 1/2A HPVs that have an established and much higher carcinogenicity.

HPV16 is highly virulent and the most prevalent type in cervical cancer. In Chapter 9, we performed a longitudinal study towards the natural history of infections by different genetic variant lineages of HPV16. HPV16 variants were individually characterized through a specifically designed assay in cervical swabs, whole-tissue sections and laser-capture micro-dissected regions of biopsies collected from women in the control arm of a vaccine trial. The HPV16 genotype consistently detected in follow-up samples usually involves a persistent infection with the same variant. We found that multiple HPV16 variants in one woman are rarely observed as opposed to the occurrence of multiple HPV genotypes.

Part 3: Application of HPV genotyping assays in clinical studies

The GP5+/6+ LMNX can serve as an alternative hrHPV detection method for the clinically validated GP5+/6+
PCR-based EIA. The benefits of read-out by the LMNX over the EIA are its high-throughput capacity, internal control for specimen adequacy, and individual identification of up to 14 hrHPV genotypes, if desired. In **Chapter 10**, GP5+/6+ LMNX demonstrated clinical performance characteristics that were non-inferior to the GP5+/6+ EIA, when performed on a validation panel of clinical specimens composed by an international consortium for clinical VALidation of GENotyping Tests (VALGENT). Based on these findings, we concluded that GP5+/6+ LMNX is a suitable stand-alone hrHPV test for primary cervical cancer screening.

**Chapter 11** describes the clinical evaluation of the FTA solid-state carrier cartridge, a novel device for the storage of self-collected cervicovaginal specimens. This device has potential utility for non-responders in nations with cervical cancer screening and for women in low-resource settings. In a cohort of women referred due to abnormal cytology, a self-collected (FTA) and a conventional physician-collected (liquid-based) cervical specimen were tested for hrHPV by SPF10 LiPA25 (version 1) and GP5+/6+ LMNX. HrHPV detection was lower in the FTA self-collected sample according to both tests. However, the combination of FTA-based self-sampling with SPF10 LiPA25 (version 1) hrHPV testing approached the clinical performance of GP5+/6+ LMNX on conventional physician-collected specimens. This underlines the need for clinical validation of the complete diagnostic process, i.e., sample collection device, storage medium, processing, HPV amplification and read-out method.

Finally, in **Chapter 12** we provide a general discussion of the findings in this thesis in the context of other studies and on future perspectives.
Chapter 13