Summary
Human papillomavirus (HPV) is one of the most common sexually transmitted pathogens worldwide. From the general population 80% will be infected at some time during life. Most people are infected with HPV soon after their first sexual intercourse.

More than 120 HPV types have been identified of which 15 are classified as high-risk (hr) or oncogenic HPV types. They belong to the α7 and α9 species. Hr HPV has now been recognised as the causative agent of cervical cancer and its precursor cervical intraepithelial neoplasia (CIN). Moreover, HPV infections are also associated with a significant number of squamous cell carcinoma of the anus, vulva, vagina, penis, mouth and oro-pharynx. HPV16 and 18 are the most common hr HPV types and responsible for 70% of all cervical cancer cases.

The mucosal surface of the genital tract provides the first line of defence against genital HPV infection. Naturally derived HPV-specific antibodies at sites where HPV infections take place may have neutralizing capacity and can inhibit the binding of HPV to cell surface receptors necessary for entering the host cells. Neutralizing antibodies may also enable viral uptake by antigen presenting cells that will lead to the destruction of the virus.

In the Netherlands the bivalent HPV vaccine (Cervarix®, containing HPV16/18 L1 virus-like particles (VLP), GlaxoSmithKline Biologicals) was implemented into the national immunization program in 2010 in a 2+1 schedule for girls 12 years of age. In addition, a catch-up vaccination campaign was initiated among girls aged 13-16 years. The bivalent HPV vaccine provides sustained protection against HPV16/18 infections over at least eight years after the first vaccination. The HPV16/18 antibody levels derived after HPV vaccination were found to be 10-100 times higher as compared to naturally derived HPV-specific antibody levels.

In this dissertation, we studied the quality, quantity and characteristics of the humoral immune response after HPV infection and after HPV vaccination.

**Antibody characteristics**

To assess humoral immune responses after HPV vaccination and infection, several serological assays have been developed. The most meaningful evaluation of the functional immune response after HPV vaccination or infection is provided by the pseudovirion-based neutralization assay (PBNA), which is the ‘gold standard’ for the measurement of HPV-specific neutralizing antibodies. However, its use in large sero-epidemiological studies or clinical trials is challenging since this assay is laborious and the production of pseudovirions is a complicated procedure. Most serological analyses of antibody responses after HPV infection have relied on assays, e.g. competitive Luminex immunoassay (cLIA), VLP-ELISA and gluthathione-S-transferase L1 based multiplex immunoassay (GST-L1-MIA) that are alternatives of the PBNA. A good correlation was observed between the PBNA and the VLP-ELISA for HPV16 and 18.

In chapter 2 we compared 3 assays for the measurement of HPV-specific serum antibody levels: the VLP-MIA, the cLIA, and the GST-L1-MIA. These assays showed good correlations for both naturally induced and vaccine-derived HPV-specific antibody levels. Although these serological assays have different assay characteristics, they can all be used for the detection of naturally derived and vaccine-derived HPV-specific antibodies. In our studies, we have
used the VLP-MIA. This assay can measure HPV-specific antibodies against 7 hr HPV types simultaneously (HPV16, 18, 31, 33, 45, 52 and 58). The VLP-MIA correlates well with the VLP-ELISA. We showed that the VLP-MIA is reproducible and that the binding of monoclonal antibodies recognizing conformational epitopes on HPV16 and 18 was type-specific.

In order to assess HPV-specific IgG characteristics, we evaluated multiple aspects, such as avidity, neutralizing capacity and cross-reactivity, of the humoral antibody response after HPV infection and vaccination, as described in chapter 3. Vaccine-derived antibodies were mainly serotype-specific and cross-reacted only partially with other HPV types within the species. Naturally derived HPV-specific antibodies from single-positive sera were, as expected, highly type-specific and tended to be neutralizing. In contrast, antibodies of multi HPV type-positive sera were less specific, cross-reactive, and tended to be non-neutralizing. Immune parameters other than antibody levels that might correlate with protection have not been defined and data on antibody characteristics such as avidity are scarce. Antibody avidity generally increases over time following encounter with an antigen and memory responses are characterized by the production of high-avidity antibodies. We determined the avidity of HPV-specific antibodies and found that the avidity of vaccine-derived antibodies was approximately 3 times higher than after HPV infection. These results imply that the avidity of HPV antibodies might be used as a potential surrogate of protection. However, further research is needed to corroborate this hypothesis.

**HPV seroprevalence**

A detectable antibody response to the L1 capsid protein develops in 50-70% of infected individuals. These naturally derived HPV-specific antibody levels are relatively stable over time. Therefore, the HPV antibody response is a measure of past HPV exposure which can be investigated in serosurveillance studies.

**Chapter 4** concerns a population-based serosurveillance study performed in 2006-07, in which we studied the age-specific seroprevalence of seven hr HPV serotypes (HPV16, 18, 31, 33, 45, 52 and 58) among the Dutch general population in a pre-vaccination era. HPV is a sexually transmitted virus and people are infected soon after first sexual intercourse. Therefore, HPV vaccination is targeted at pre-adolescents. This age is also justified by the results we found. A step-up in HPV seroprevalence of women and men is observed at ages of sexual debut around 15-19 years. This increase in HPV seroprevalence was most pronounced in women. Relatively constant HPV seroprevalences were found in the middle aged cohorts and the HPV seroprevalence slightly decreased in the elderly.

HPV seroprevalences in the pre-vaccine era (12-16 years) can be used as baseline to evaluate long-term population effects of HPV16/18 vaccination. We can monitor for instance the effect of vaccination on virus circulation in both women and men, possible type replacement and herd immunity after HPV vaccination. Changes in HPV seroprevalence among the Dutch general population of the seven hr HPV types, identified by our VLP-MIA between 1995-96 and 2006-07 are described in chapter 5. HPV antibody seroprevalences in the general population have increased in the Netherlands with 3.1% over that 11-year period due to a significant higher
seroprevalence for HPV16, 18, 31 and 45. HPV16 seroprevalence among young women shifted to younger ages (from 20-24 to 15-19 years), probably due to changes in sexual behaviour over that period, in particular the age of sexual debut. Interestingly, an increase in HPV seropositivity in older aged individuals (>40 years) was also observed between these two time periods and might be due to an increase in sexual activity among older aged individuals because of the development of new therapeutic interventions that can be used when having sexual problems, especially in men. Sexually active older women might be more vulnerable to sexually transmitted infections because of post-menopausal changes. These post-menopausal changes can cause atrophy or thinning of mucosa with decreased lubrication that can result in small lesions of the cervix and facilitate the entry of pathogens when these women are sexually active. These age groups are thus more at risk for sexually transmitted infections.

**Vaccine-derived immune responses**

Together with the vaccine implementation, the HAVANA (HPV Amongst Vaccinated And Non-vaccinated Adolescents) study was designed to assess the early effects of the vaccination program. In this study, young girls (14-16 years of age) were enrolled expected to become sexually active in the next years post-vaccination. By taking yearly cervical swabs, blood and cervical secretion samples (CVS) from these girls we were able to monitor HPV-DNA status in cervical swabs and to relate these results with the presence of HPV-specific antibodies in serum and CVS. By the combination of these parameters we are able to monitor in these young adolescents the expected age-related HPV-DNA increase associated with increased sexual activity. Monitoring the type specific HPV-DNA prevalence in cervico-vaginal secretions and vaccine-induced HPV-specific antibody responses in serum and CVS before and shortly after introduction of routine HPV16/18 L1 vaccination offers the opportunity to evaluate early effects of the vaccination program.

As expected, the HPV-DNA prevalence among young girls prior to vaccination was low (chapter 6). Of the hr HPV-DNA positive girls approximately one third were HPV antibody seropositive for at least one of the seven HPV types tested, and almost 80% of these girls had HPV-specific antibodies of the corresponding HPV type. In the near future, within this study population acquisition of new HPV infections will be evaluated in relation to their vaccination status, serostatus and risk factors for HPV (sero)positivity will be studied longitudinally.

The bivalent HPV16/18 vaccine induces high antibody concentrations in serum while data about antibody responses in CVS are limited. In chapter 7 we describe the pre- and post-vaccination IgG and IgA antibody responses against seven hr HPV types in CVS and serum. After vaccination, HPV16 and 18 specific IgG and IgA antibodies were detectable in CVS and these antibody concentrations correlate well with serum antibody levels, although IgA levels were much lower as compared to IgG levels. The correlations of HPV16 and HPV18 IgG and IgA antibody levels between serum and CVS might denote that vaccine derived antibodies transudate and/or exudate from the systemic circulation to the cervical mucosa although other
immune mechanisms cannot be excluded. HPV-specific antibody levels in CVS were lower as compared to serum, however, these antibody levels remained constant up to two years post-vaccination and might therefore contribute to a protective environment at the cervix. This hypothesis is supported by the high correlation between the antibody levels of tetanus toxoid and diphtheria toxoid in serum and CVS because these vaccine-induced antibodies are not produced at the cervical matrix. Transudation and exudation of HPV-specific antibodies probably contribute to sufficient antibody levels at sites where HPV infections actually take place and therefore may provide protection against HPV infection and/or re-infections.

Effective and safe vaccination is essential in individuals with impaired immunity caused by chronic disorders, such as juvenile idiopathic arthritis (JIA) and systemic lupus erythematosus (SLE), because these individuals are at increased risk of infections due to the immunosuppressive effect of the disease or the immunosuppressive treatment. Arthritis patients with SLE have an increased risk of multiple and persistent HPV infections leading to a higher prevalence of CIN compared to healthy individuals. In chapter 8 and 9 we describe the immunogenicity and safety of the bivalent HPV vaccine in girls with JIA, SLE, juvenile dermatomyositis, or overlap syndromes and compared these with healthy female adolescents aged 12-18 years. The HPV vaccine is safe and immunogenic in the 12 months after the first HPV vaccination, but the magnitude of antibody responses and memory B-cell responses appeared to be lower in patients compared with healthy controls. Since long-term protection against HPV infection might be inadequate in JIA patients, we recommend surveillance of HPV-specific antibody concentrations and next to HPV vaccination also frequent secondary prevention via cervical smears. Also in patients with arthritis-like disease based on SLE, juvenile dermatomyositis, or overlap syndromes the HPV vaccine appeared less immunogenic in comparison to healthy girls. These results warrant a larger prospective study to unravel the mechanisms behind these poor humoral immune responses.

### Importance of HPV serology

In chapter 10 the findings of this thesis and the importance of serology in future HPV research are discussed. HPV-specific seroprevalence studies are necessary to monitor the vaccine-derived protection against HPV and to generate information on groups at risk for HPV infections, herd immunity after HPV vaccination and possible type-replacement in vaccinated individuals. Therefore, it would be advisable to perform a subsequent serosurveillance study in which the effect of HPV vaccination can be monitored, for instance 10 years after the serosurveillance study performed in 2006-07. Next to the collection of sera in these serosurveillance studies, the collection of cervical DNA among HPV vaccinated or non-vaccinated adolescents is of interest to investigate the impact of the HPV vaccine on HPV repertoire, possible HPV type replacement and vaccine efficacy.

Currently, the HPV vaccination is targeted only at young girls. However, boys might also benefit directly from HPV routine vaccination as it is now recognized that HPV can cause squamous cell carcinoma that affect men (anus, penis, mouth, oro-pharynx). Important for this debate is
the magnitude of herd immunity that will be conferred to men from HPV vaccinated women. Herd effects increase with higher vaccine coverage. However, in the Netherlands HPV vaccine coverage amounts only just over 50% among young girls and in case the vaccine coverage will not increase over time, boys might benefit only from direct HPV vaccination. In addition, vaccinating boys may have a stimulating effect on the HPV vaccine coverage among girls, as opponents of (HPV) vaccination indicate the vaccination of only girls as gender discrimination.

In this thesis we showed that HPV serology is of great added value for investigating the immune responses after HPV infection and for monitoring the effects of HPV vaccination. The measurement of HPV-specific naturally induced antibodies is important to provide information about trends in HPV seroprevalence over time and to monitor past HPV infections. Investigating the humoral immune responses among vaccination target groups has shed light on the immunological mechanisms that contribute to detectable and protective HPV-type specific antibody levels at the cervix, originally induced in the systemic circulation. In addition, assessment of the humoral immune response will provide insight in the immunological effects of HPV vaccination individually (protection against HPV) and for the whole population (possible induction of herd immunity) which will provide valuable information for the prevention of cervical cancer.