Summary

*Chlamydia trachomatis* is the most prevalent bacterial sexually transmitted disease, affecting in the Netherlands 60,000 persons each year. The majority of Ct infections remain asymptomatic in men (50%) and women (70%), contributing to further transmission and the development of late complications, like pelvic inflammatory disease. *Chlamydia trachomatis* can be divided into 19 serovars, historically based on epitopes of the Major Outer Membrane Protein (MOMP). Nowadays, serovars are determined by sequences of the *Omp1* gene (encoding MOMP). The different serovars are associated with various disease types. In general, the serovars A-C cause ocular disease (trachoma), the serovars D-K occur as anogenital infections and the serovars L1-L3 plays a role in development of invasive disease lymphogranuloma venereum (LGV). For clinicians, differentiation between an LGV serotype and a anogenital (D-K) serotype is important, since prolonged antibiotic treatment is necessary in case of an LGV infection. In previous studies an association between a *Chlamydia trachomatis* co-infection and an elevated risk to have a persistent Human Papillomavirus infection or to develop cervical cancer was observed, although the statistics in those studies were suboptimal. From these observations it can be concluded that validated *Chlamydia trachomatis* detection and genotyping assays might be of major importance for both researchers and clinicians. In the current thesis, two new *Chlamydia trachomatis* detection and genotyping assays were evaluated (part 1) and used for epidemiological serovar studies (part 2), LGV studies and studies that investigate the cofactor role of *Chlamydia trachomatis* (Ct) in the development of (pre)cancerous cervical lesion (part 3).

Part 1

Several commercially available Ct detection assays are available, but no Ct genotyping assays. Ct genotyping is important in research settings regarding the epidemiology, the phylogenetics and transmission patterns. For the clinician, Ct genotyping is also important to differentiate LGV Ct infections from non-LGV Ct infections. In the current thesis, a new PCR based Ct Detection and genoTyping assay (Ct-DT assay) is described, using the specific algorithm of Ct detection with a DNA enzyme immunoassay (DEIA), followed by Ct genotyping with a Reverse Hybridization Assay (RHA) that differentiates between the 14 major serovars (A, B/Ba, C, D/Da, E, F, G/Ga, H, I/la, J, K, L1, L2/L2a and L3) (Chapter 2). For Ct DNA amplification, the Ct-DT DEIA and Ct-DT RHA need the same broad-spectrum multiplex PCR, which is a dual target PCR amplifying a 89 bp amplicon of the endogenous plasmid and a 157/160 bp amplicon of the *Omp1* gene. This PCR is completely specific for
the species *C. trachomatis*. As a first step in the diagnosis the PCR products will be screened for Ct with the Ct-DT DEIA. The developed Ct-DT DEIA showed an excellent agreement with other Ct detection assays, like the COBAS TaqMan (Roche Diagnostics; Chapter 2) and the Hybrid Capture 2 Ct assay (Qiagen; Chapter 3), making the Ct-DT DEIA a good alternative for routine Ct detection. Besides an assay for routine Ct detection, the Ct-DT DEIA is a selection method of Ct positive PCR products that can be further genotyped with the Ct-DT RHA. For decades, Ct genotyping was performed in research settings to get more insight into the Ct serovar distribution, the relation of Ct serovars with signs and symptoms, the phylogenetics and Ct transmission. Since the ongoing epidemic of LGV among homosexual men, genotyping results are also interesting for clinicians. Until recently, sequencing of the *Omp*1 gene was used as the reference method. However, the newly developed Ct-DT RHA is a quick and sensitive alternative for *Omp*1 sequencing (Chapter 4). Compared to direct *Omp*1 sequencing, the Ct-DT RHA is faster and can simultaneously identify multiple serovar infections. This makes the CT-DT RHA more suitable for large scale serovar distribution studies. However, *Omp*1 sequencing has a higher discriminatory power since it can detect new point mutations in the *Omp*1 gene, which could be important in phylogenetic studies or Ct networking studies (Chapter 4). To further increase the high throughput possibility for Ct genotyping, the Ct-DT RHA assay was transformed to a Ct Microsphere Suspension (MS) assay (Chapter 5) utilizing the xMAP Microsphere Suspension Array Technology (Luminex Corporation, Austin, Texas). Both, the Ct-MS assay and the Ct-DT RHA, uses the same PCR products. Since an almost perfect agreement between the Ct-MS assay and the Ct-DT assay was observed, it could be concluded that the Ct-MS assay is a good alternative for the Ct-DT RHA, due to its higher throughput. Nevertheless, for the Ct-MS assay the laboratory settings require a Luminex platform, while the Ct-DT RHA could be performed manually. Thus at this moment two new Ct detection and genotyping assays are available that can be easily used in further research studies, and also in routine diagnostics.

**Part 2**

Several serovar distribution studies were performed in different areas over the world. Besides a serovar distribution for a specific geographic area, those serovar distribution studies can reveal associations with clinical symptoms. Although the majority of the studies observed that the serovars E, F and D account for 70% of all serovars, different genotyping methods were used which can clearly interfere with the outcome of the serovar distribution. In the current
thesis we determined the serovar distribution with the Ct-DT RHA for Ct positive women in Uganda (n=53; Chapter 2), in Costa Rica (n=827; Chapter 3), Ct positive men and women in Russia (n= 181; Chapter 6), and in the Netherlands (n= 433; Chapter 7). Overall, the serovar distribution was more or less the same for the different countries with serovar E as the most prevalent serovar. The only exception was the distribution observed in Uganda, in which serovar G/Ga was the most frequently observed serovar. Regarding double serovar infections, we could conclude that 2-4% of the individuals infected with Ct are infected with more than one serovar. Also, an increased Ct antibody response in the blood was observed among individuals infected with a serovar that belongs to serogroup B (Chapter 9). This could be a sign of a more severe inflammation for serovars belonging to serogroup B, but the exact role has to be further investigated. In Chapter 8, we determined the serovar distribution in rectal swabs from 100 non-LGV Ct positive homosexual men and observed a different serovar distribution compared to the heterosexual population. As shown in other serovar distribution studies among homosexual men, the serovars G/Ga, D/Da and J were the most prevalent serovars. The occurrence of other prevalent serovars in this group might be an indication for a closed homosexual community or a rectal tissue tropism of specific serovars.

It can be concluded that the worldwide the serovar distribution is geographical relatively stable. However, homosexual men have a different serovar distribution pattern.

Part 3
Co-factors might affect the risk of progression from infection with carcinogenic HPV to cervical precancer and cancer. Some, but not all, studies have shown Ct to be a risk factor for cervical cancer. It is not sure whether this increased risk is causal or due to residual confounding caused by inadequate adjustment for HPV infections. In Chapter 10, we investigate the possible co-factor role of Ct in the development of (pre)cancerous lesions of the cervix. The analyses conclude that Ct was not a risk factor for the development of precancerous lesions, but more a marker for a high risk HPV infection. Also, the endocervix (glandular epithelium) is the major site for a Ct, infection while all epidemiological associations were observed between Ct antibodies in serum and the development of squamous cell carcinoma and not adenocarcinoma. In Chapter 11 we investigated whether Ct DNA was detectable in cervical adenocarcinomas (n=71). In none of the tumors Ct DNA could be detected, which is in line with previous studies. From these studies it can be concluded that Ct is not a risk factor for the development of cervical cancer, but a marker for high risk HPV infections.
In summary, two new genotyping techniques were evaluated that can contribute to more efficient Ct research and Ct diagnostics. The serovar distribution worldwide is relatively stable, although minor differences are observed. Further, an association between serogroup B and an elevated Ct antibody response in the serum is observed. Finally, Ct infections earlier in life are not a risk factor for the development of cervical cancer, but are a marker for a High Risk HPV infection.