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Karimi, O.

2011

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### ***citation for published version (APA)***

Karimi, O. (2011). *Immunogenetics of infection and inflammation of the urogenital and gastrointestinal tracts and probiotics*.

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## Chapter 1



### **Epidemiology, diagnostics, and treatment of urogenital *Chlamydia trachomatis* infections**

O. Karimi, A. Catsburg, H.J.C. de Vries, H.S.A. Fennema, S. Ouburg, M.A. van Agtmael, P.H.M. Savelkoul and S. A. Morré



## Summary

*Chlamydia trachomatis* is the most prevalent cause of sexually transmitted bacterial infections in The Netherlands. In women, it is an important cause of pelvic inflammatory diseases, tubal pathology, infertility, ectopic pregnancy, and chronic abdominal pain. Vertical transmission from mother to child during vaginal delivery may cause neonatal infection such as conjunctivitis and pneumonia. In men, this infection may cause urethritis and in rare cases chronic prostatitis and/or epididymitis. The infection is asymptomatic in 80% of women and 50% of men and therefore untreated in most cases. This results in a huge reservoir for transmission of the disease. Recent studies show an increase in the prevalence of *Chlamydia trachomatis* infections in The Netherlands, especially in Groningen and Amsterdam, a discovery based on focused case-finding. New and more sensitive tests make early detection and treatment of patients possible. This review will elaborate on the epidemiology, diagnostics, and treatment of *Chlamydia trachomatis* infections.

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## Introduction

*Chlamydia trachomatis* (Ct) is the most common bacterial cause of sexually transmitted diseases (STD) with an incidence of 60.000 out of 110.000 reported STD's in the Netherlands.<sup>1</sup> The new annual results of STD centres in Groningen and Amsterdam report an increase of Ct infections, especially in adolescents and young adults.

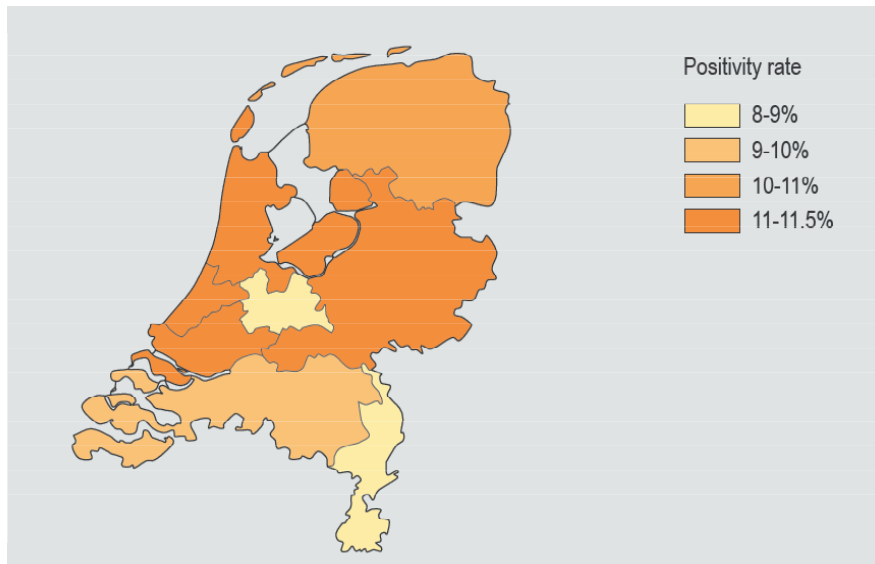
The disease course is asymptomatic in 80% of women despite the fact that long-term complications may occur. Clinical manifestations of the infection differ from cervicitis to 'pelvic inflammatory disease' (PID) and tubal pathology. Vertical transmission often leads to neonatal infections such as conjunctivitis and pneumonia. In men Ct causes urethritis, and rarely chronic prostatitis, epididymitis, or both. Ct-infections are treatable, since the introduction of molecular detection methods in the early nineties relatively simple to detect. It is therefore important to detect and treat Ct-infections at an early stage in order to prevent late complications and further spreading of the disease. This review will discuss the epidemiology, diagnostics, and treatment of uro- and anogenital Ct-infections.

### *Epidemiology of the disease*

Annually, an estimated 33.000 women are diagnosed with a *Chlamydia* infection (1). Based on information mainly from symptomatic infections, it is estimated that 8-20% of Ct-infections result in PID.<sup>2-6</sup>

Higher prevalences of Ct have been reported in urban areas (3,2%) compared to the rural areas (0,6%). Ethnic minorities (Surinamese/Antillean 8,2%; Turkish/Moroccan 3,1%) also show a higher prevalence compared to native Dutch (1,8%). The same applies to people with a lower education (2,1%) compared to persons with a higher education (1,1%).<sup>7</sup> Ct consists of different serotypes that cause infections with a different clinical course. Ct-serotypes A, B, and C cause trachoma and will not be discussed here. Serotypes D - K cause urogenital infections with a mild course due to the fact that they infect only the mucosal epithelium (Figure 2).

Serotype L however, is more aggressive and causes the more invasive lymphogranuloma venereum (LGV) infections, where the deeper layers get infected and which also involve the lymph nodes causing severe immunologic infections.



**Figure 1.** *Chlamydia trachomatis* positivity rates based on the STD report in the Netherlands in 2009. Figure courtesy of the National Institute of Health and the Environment (RIVM).<sup>62</sup>

Ct-serotype L in the Netherlands is predominately found in men who have sex with men (MSM). Till 2003 LGV was almost exclusively seen in the Netherlands as an imported STD from LGV-endemic sites.<sup>8</sup> Since the LGV outbreak in Rotterdam in 2003 however, a large LGV-reservoir appears to exist in the Netherlands and outside. Even in 2009-2010, new cases of LGV were found at the STD outpatient clinic of the Public Health Services of Amsterdam.<sup>9</sup> About 50% the reported Ct-infections at the Public Health Services of Amsterdam are from asymptomatic infected individuals. A large part of these are people who get tested for STDs at the start of a new relationship. A proper registration of the reasons why people get tested may be of importance to get insight in behaviour and corresponding risk to get the infection. However, registration of these reasons is not yet uniform in the Netherlands.

### ***Clinic***

The clinical course shows a remarkable interindividual diversity in transmission of the infection, the symptoms, persistence or clearance of infection, and development of late complications.<sup>14-16</sup> In 80% of women and 50% of men the infection develops asymptotically and no treatment will be sought.<sup>10-13</sup> In 45-65% of asymptomatic cases transmission has been described from the index patient to the partner.<sup>17-19</sup> The transmission frequency in symptomatic Ct-infections is significantly higher (65-90%). The incubation period of *Chlamydia*-infections is difficult to determine. In symptomatic infections an incubation period of 2-3 weeks has

been mentioned. When there are no complains, the incubation period is not known. Some patients clear infection spontaneously, sometimes within one month, whereas others may be asymptotically infected for up to 3 years. Persistence of infection and re-infection are difficult to distinguish from one another, especially when the causative serovars are identical.<sup>14,15,20,21</sup> There is no clear explanation for this heterogeneity in susceptibility to Ct-infection and disease progression after similar exposure to bacteria.

**Table 1:** Clinical manifestations of *Chlamydia trachomatis* infections in adults, children, and newborns.

	<b>Men</b>	<b>Women</b>	<b>Newborns and children</b>
Pathology:	<b>Urethritis</b> <b>Epididymitis</b> <b>Proctitis</b> <b>Prostatitis</b> <b>LGV<sup>2</sup></b>	<b>Urethritis</b> <b>Cervicitis</b> <b>Endometritis</b> <b>Salpingitis en PID<sup>1</sup></b> <b>Periappendicitis</b> <b>Perihepatitis or FHC<sup>3</sup></b> <b>Perisplenitis</b> <b>LGV<sup>2</sup></b>	<b>Conjunctivitis</b> <b>Pneumonia</b> <b>Pharyngitis</b>
Sequelae:	<b>Urethral stricture</b>  <b>Infertility</b> <b>Reiter's syndrome</b>	<b>Ectopic pregnancy</b>  <b>Infertility</b> <b>Reiter's syndrome</b>	<b>Obstructive pulmonary disease</b>

1= PID: Pelvic Inflammatory Disease, 2= LGV: Lymphogranuloma Venereum, 3 = FHC: fitz-hugh-curtis syndrome.

Factors important for this diversity may be: bacterial factors, host factors, environmental- and epidemiologic factors, coinfections, but also the combined interaction of these factors. Contribution of these different factors and their consistency is being investigated by an international, EU-funded *Chlamydia* consortium ([www.EpiGenChlamydia.EU](http://www.EpiGenChlamydia.EU)).

### ***Clinic in women***

Women frequently undergo an asymptomatic infection, but in symptomatic cases complaints may vary from urinary tract infections and metrorrhagia to vaginal discharge and, in some cases, pyuria, and pain or loss of blood during intercourse. Furthermore, lower abdominal complaints are frequent and ill-understood.

Consecutive or multiple symptomatic Ct-infections are associated with serious pathology of the pelvis. Serious abdominal complaints usually give rise to the first suspicion of PID. This is an acute infection of the upper urogenital tract structures in women in which the uterus, tubae, and ovaries may be affected, with as consequence endometritis, salpingitis, and tubo-ovarian abscess. Ectopic pregnancies and tubal infertility may also occur.<sup>22</sup>

Furthermore, infections may ascend to the abdominal cavity and cause a sterile peritonitis with ascites. Acute perihepatitis (fitz-hugh-curtiss syndrome) was initially only attributed to gonococcal infections, but the frequency appears to be higher in patients positive for Ct.<sup>23-25</sup> In about 5% of acute salpingitis cases, early laparoscopy reveals perihepatic inflammation reaching from oedema and erythema of the hepatobiliary capsule to exsudate with fibrinous adhesions between the parietal and visceral peritoneum, leading to the characteristic “violin strings”.<sup>26,27</sup> Peri-appendicitis and perisplenitis have also been reported.<sup>28,29</sup> Chronic Ct-infection is also associated with foetal death, preterm birth due to early rupture of membranes, low birth weight, and puerperal fever. Finally, untreated Ct-infections are associated with a cumulative risk of 72% for the development of an early or late PID in the first 24 months after abortion (*Table I*).<sup>30</sup>

### ***Clinic in newborns***

Newborns that got a Ct-infection during birth develop conjunctivitis and/or pneumonia. In severe cases this infection may lead to blindness or even death of the neonate.

### ***Clinic in men***

Ct-infections in men have a more symptomatic course than in women. Urethritis is the most common symptom in men. Urethritis is usually classified as gonococcal versus non-gonococcal urethritis. Approximately 10% of the gonococcal infections and up to 40% of the non-gonococcal urethritis do not give symptoms. In symptomatic non-gonococcal infections symptoms include dysuria and urethritis with mucoid or watery discharge. Long-term complications are chronic prostatitis and epididymitis, together with urethra strictures and male infertility.<sup>31</sup> In addition, Ct infection increases the risk to develop Ct-induced arthritis (Reiter’s syndrome).<sup>32</sup>

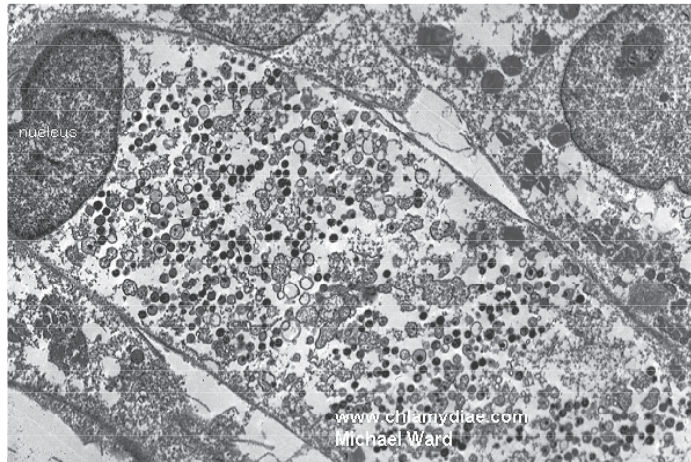
LGV has a severe disease course. It is often an invasive and ulcerative disease, which, after the infection breaks through the mucosal layer, spreads in the connective tissue layer and progresses to locoregional lymph nodes via lymphatics where it causes destructive and systemic inflammatory reactions.<sup>33</sup> LGV may cause an anorectal syndrome, marked by severe proctitis with tenesmus, pain, bloody discharge, and constipation due to local oedema. Untreated it may cause irreversible anal strictures with pain, ‘soiling’, constipation, and megacolon.

**Diagnostics**

Ct-inclusion bodies were first demonstrated with giemsa staining in 1907.<sup>34-35</sup> Since then the diagnostics of Ct-infections have improved tremendously with regard to sensitivity, specificity, time per assay, and laboratory standardisation. The technical development in Ct-detection from culture, to enzyme-immuno-assay (EIA) and direct fluorescent antibody-assay (DFAA) to recent nucleic acid amplification assays (NAAA) will be described. Initially, culture was considered the golden standard for the detection of Ct.<sup>36,37</sup> Development of monoclonal antibodies to directly detect Ct in clinical samples was a major breakthrough.<sup>38,39</sup> In 1970 Wang and Grayston were the first to develop serological tests with which Ct could be subdivided in different serovars.<sup>40</sup> With this test serovar specific antigens could be used to detect anti-*Chlamydia*-antibodies in serum or in tears.

Commercially available tests such as Ct-EIA, SeroCt, and Ct-pELISA make the otherwise difficult serology easier and more accessible.<sup>41</sup> Yet, serology is only useful at some indications: diagnostics of LGV or, in case of (female) infertility, to detect a Ct-infection in the past.<sup>42,43</sup> Direct hybridisation probe tests use DNA/RNA or DNA/DNA hybridisation probes, generating a signal without target amplification. In the Netherlands two tests are being used where the technique is based on signal amplification: target amplification. The Gene Probe PacePACE-2 (Gen-Probe, San Diego California) uses rRNA, which is available in high numbers per cell, as a target.<sup>44</sup> The Digene Hybrid Capture II test-Test (HCII, Digene, Gaithersburg, Md) uses signal amplification.<sup>45</sup> Over 10 years ago, the first commercial diagnostic nucleic acid amplification tests (NAAT) came on the market. Apart from the so-called in-house-PCR-assays there are four large commercial tests on the market: COBAS Amplicor<sup>®</sup> (PCR technique, Roche) and the newer versions of TaqMan & TaqMan 48, and the most recent COBAS X 4800 system with automatic sample preparation system, the BD-ProbeTec<sup>®</sup> ('strand displacement assay'; SDA; Becton-Dickinson), APTIMA<sup>®</sup> Combo 2-assay ('transcription mediated amplification assay', GenProbe) with as newest assay the TIGRIS, and finally the Abbott LcX<sup>®</sup> ('ligase chain reaction', Abbott Laboratories) which has recently been replaced by the Abbott m2000.





**Figure 2:** Human cell filled with *Chlamydia trachomatis*. Electronmicroscopic photograph courtesy of Prof. Michael Ward [www.Chlamydiae.com](http://www.Chlamydiae.com)

The sensitivity of different tests is significantly better than previously described techniques (Table 2), and the specificity of these tests is also very high.

This means that 25-50% more infections are detected compared to the classical cell culture for *Chlamydia*. The current generation NAAT-systems have taken into account the reliability of the generated outcome in different ways, both with regard to the false positives and with regard to the inhibition problem by taking an internal control in the analysis of every sample. The chance of contamination has been greatly reduced by using closed systems. False negatives due to wrong sampling, transportation problems, and sampling from a non-infected anatomical site, while the patient is positive (cervical vs. urethral) are variables that can't be changed by test techniques.

More recently some rapid tests, the Point-of-Care (POC)-tests, have been released.<sup>46-48</sup> These tests are easy to perform and could detect a Ct-infection within half an hour. The reported sensitivity and specificity however are appalling. For instance, in comparison with PCR the Clearview *Chlamydia* MF Rapid seems to have a sensitivity and specificity of 32,8 and 49,7 % respectively.<sup>48</sup> The Biorapid *Chlamydia* Ag test, the Quick Vue *Chlamydia*, and the Handilab-C test scored even worse. The sensitivity of these tests is respectively 17, 27, and 12%. The use of these kinds of tests leads to false reassurance. Given the poor quality of these POC-tests, it was proposed last year to strongly discourage their.<sup>50</sup> Introduction of this kind of tests may endanger the reduction of the *Chlamydia* problem.

**Table 2:** Sensitivity and specificity of *C. trachomatis* detection assays (\*).

Test	Sensitivity (%)	Specificity (%)	Detection limit (no. organisms)
NAAT <sup>a</sup>	90-95	>99	1-10
DFA <sup>b</sup>	80-85	>99	10-500
EIA <sup>c</sup>	60-85	99	500-1000
DNA-probe <sup>d</sup>	75-85	>99	500-1000
Cell culture	50-85	100	5-100
POC <sup>e</sup>	25-55	>90	>10000

(\*) = based on Bianchi et al., 1998 and adapted from Land et al., 2009, a = Nucleic Acid Amplification Test.<sup>60,61</sup> DNA-based: PCR AmpliCor assay (Roche Diagnostics, Basel, Switzerland), LCR (Abbott Laboratories, Abbott Park, Illinois, USA), recently replaced by the Abbott m2000rt; SDA (Becton Dickinson, Franklin Lakes, New Jersey, USA), current assay is the BD ProbeTec. RNA-based: TMA, AMP-CT (Gen-Probe, San Diego, California, USA), The currently most used assay of Gen-Probe is the TIGRIS, b = Direct Fluorescence Assay. Syva MicroTrak (Syva Co, Palo Alto, California, USA), c = Enzyme Immuno Assay. Vidas (BioMérieux, Craponne, France), d = DNA-based: Hybrid capture assay (Qiagen, Hilden, Germany), AmpliProbe system (ImClone Systems, New York City, New York, USA); RNA-based: PACE 2 assay (Gen-Probe, San Diego, California, USA), e = Point-of-Care test. Handilab-C (Zonda Incorporated, Dallas, Texas, USA), Biorapid Chlamydia Ag test (Biokit, Barcelona, Spain), QuickVue Chlamydia test (Quidel Corporation, San Diego, California, USA).

## Treatment

### Medical treatment

Cervical and urethral Ct-infections are preferably treated with one single dose of 1 gram azithromycin orally, or doxycycline 100mg twice daily during 7 days. Also in case of pregnancy, azithromycin is preferred over amoxicillin (three times a day during 7 days) or erythromycin. From a meta-analysis where the effectiveness of a single dose azithromycin was compared to that of erythromycin and amoxicillin in pregnant women, no difference was observed. An additional advantage of azithromycin, next to the promotion of compliance, is that it has less adverse events than erythromycin and amoxicillin.<sup>51</sup>

In case of allergic reactions to azithromycin or doxycycline, amoxicillin is being advised in a dosage of 500 mg three times a day during 7 days. This course needs to be completed without interruption. So far, no resistance has been reported for the medication used against Ct-infections. To prevent transmission, the current partner(s) need(s) to be treated as well. In case of complications like PID or epididymitis, given the possibility of mixed infections, longer treatment is advised with multiple antibiotics. The exact medication and dosage depends on the microorganisms involved in the mixed infections. Treatment of LGV consists of doxycycline 100mg twice daily during 21 days or, in case of pregnancy or allergy, erythromycin four times a day 500mg during 21 days. In case buboes have already formed, they will have to be relieved to prevent suppuration and fistulising.

R1 Because of persisting complaints, LGV-infections often have to be treated for longer than  
R2 21 days. De Vries *et al.* recently showed that Ct-serotype L organisms could survive a  
R3 continuous exposure to Doxycyclin for a longer period than Ct-serotypes D-K. This stresses  
R4 the importance of a longer eradication Ct-infections (21 days instead of 7 days doxycyclin),  
R5 to treat LGV-proctitis.<sup>52</sup>  
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### R7 ***Follow-up***

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R9 This is done to exclude treatment failure, when the first-choice treatment with azytromycin  
R10 or doxycyclin was not given, or when there is doubt about compliance. Follow-up is done by  
R11 detection of reinfection and occurs by repeating the Ct-NAAT of the infected site after at least  
R12 4 weeks after completing treatment. Before then follow-up is not useful, because of the false  
R13 positive results when using NAAT.<sup>52-53</sup>

R14 In LGV, there is always follow-up after completing treatment to evaluate the given therapy  
R15 and to assess possible persisting complaints or disorders, and if necessary treatment is  
R16 continued.

R17 In case of deterioration of complaints or complications in the form of strictures, oedema, or  
R18 fistula the patient has to be sent to the internist or gastroenterologist.

R19 Several studies have shown that despite appropriate treatment, contact tracing, and partner  
R20 warning, reinfection percentages above 10% are found. Women who are at high risk (among  
R21 others those with multiple sexual partners and unsafe sexual behaviour) are recommended to  
R22 have a check up for STDs including Ct, every year. Furthermore, follow-up may be indicated  
R23 on emotional grounds (for example for reassurance).  
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### R25 ***Screening***

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R27 Apart from primary prevention, secondary prevention is an additional strategy to avoid  
R28 complications and to fight further spreading in the population. The cost-benefit effect of  
R29 screening has been investigated in sexually active visitors of the Amsterdam general  
R30 practices.<sup>54</sup> It was concluded that from farmaco-economic perspective a Ct-screening  
R31 program for sexually active women in Amsterdam is recommended. This has not been  
R32 widely implemented yet. Following the PILOT-Ct-study, in which 12000 visitors of Public  
R33 health Services in the age of 15-29 years were included in the period of September 2002  
R34 - March 2003, selective screening was recommended based on a risk profile.<sup>54</sup> Risk factors  
R35 are: changing partners, STD-related complaints, intercourse without condom, living in urban  
R36 area, age below 20 years, being of Surinamese or Antillean descent, and having a low level of  
R37 education.<sup>55</sup> Recently, dr. J. van Bergen has given an overview with regard to the screening of  
R38 urogenital Ct-infections.<sup>56</sup> In April 2008, the national *Chlamydia*-screeningimplementation  
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(CSI)-project started in high-risk populations in the regions of Amsterdam, Rotterdam, and southern-Limburg. This project includes a differentiated implementation of *Chlamydia* screening in 315.000 younger adults at the age of 16-29 years. They are invited by letter to request online a *Chlamydia*-test kit for free. Men send a urine sample to the laboratory. Women can chose between sending a vaginal swab or urine sample.

Those who are positive for *Chlamydia* are referred to the general practitioner and are treated with short course of antibiotics. Furthermore, they are asked to inform their partners and to refer them for treatment. After one year, all participants will be invited once again for a *Chlamydia*-test. This way, the effectiveness of repeatedly *Chlamydia*-screening is being tested to finally take a decision on how to the national screening should be introduced.

### **Prevention**

Sexually transmission of Ct-infections can be greatly reduced by the use of condoms, but depending on the sexual activities use of condom may prevent pregnancy but not Ct infection. Due to the high transmission percentages between partners, both in symptomatic and asymptomatic Ct-infections, STD-care cannot be complete without partner notification and treatment. This is mainly the role of the local public health care and STD centres. The use and giving of warning strips and written information may be supportive in warning of partner(s).

In symptomatic Ct-infections, all sexual partners from the 4-6 weeks before complaints are warned. In asymptomatic cases this concerns all partners in the 6 months before complaints. When it is clear that the infection must already have existed for a longer period of time (e.g. no sexual contact in last six months), and if possible, partners from further back in time are warned.

It is known that Ct-infections may persist asymptomatic for multiple years, even longer than four years.<sup>57-59</sup> It is important to explain this to both the index partner and the partner, to avoid misunderstanding in steady relationships. After starting treatment with azytromycin, sexual contact is discouraged during the first week. When treating with other antibiotics, sexual contact is discouraged until after completion of therapy.

### **Conclusion**

The increasing prevalence of Ct-infections and the magnitude of complications of undetected and untreated disorders form a degree of urgency to actively, direct, and in an early stage screen and treat. Research shows that directed screening of young women for urogenital Ct-infections decreases the incidence of PID and Ct-associated complications of reproduction such as infertility, extra-intestinal pregnancy, and chronic abdominal pain.

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Public health care and general practitioners play an important role in active screening. A first step has already been made by the CSI-project that started in April 2008. We hope that by this the incidence will decrease and thereby also the late complications at the individual level and the level of the public health.

***Instructions for practice***

1. The number of diagnosed *Chlamydia trachomatis*-infections is still increasing.
2. Nucleic acid amplification tests (NAATs) are the diagnostic tests of choice.
3. Point-of-Care tests (POCs) are not suitable for diagnostics of urogenital *Chlamydia trachomatis*-infections in the current format.
4. The recently started *Chlamydia* Screening Initiative (CSI) is of importance to reduce prevalence and transmission and thereby to fight the long-term complications of infection.

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