Andreas Edberg

PCR detection and prevalence of *Mycoplasma genitalium*
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To my loving family
Chlamydia and gonorrhea are major causes of sexually transmitted infections (STI) in adolescents worldwide. The infections are caused by *Chlamydia trachomatis* or *Neisseria gonorrhoeae*, bacteria with clinical manifestations such as urethritis, prostatitis and epididymitis among men, and urethritis, cervicitis and upper genital tract infection (i.e. pelvic inflammatory disease) among women. However, in many cases of genital tract infection, the etiology remains uncertain. In light of this, *Mycoplasma genitalium* was somewhat accidentally isolated in 1980 after prolonged incubation of urogenital specimens from men with non-gonococcal urethritis. Following the initial isolation in 1980, repeated attempts have been made to recover this extremely fastidious organism from clinical samples by culture techniques, but isolates have been rare and difficult to obtain. With the development of PCR methods in the early 1990s, detection of *M. genitalium* infection became more feasible.

The aim in paper I was to compare three different PCR assays (conventional and real-time 16S rRNA gene PCR as well as real-time *Mycoplasma genitalium* adhesin protein (MgPa) gene PCR) for detection of *M. genitalium*. The study also determined the prevalence of *M. genitalium*. Clinical specimens collected from STI attendees, 381 men and 298 women, were used to determine the prevalence of *M. genitalium* and 213 of these specimens were used in the PCR comparative study. The prevalence of *M. genitalium* infection in men and women was 27/381 (7.1%) and 23/298 (7.7%) respectively. In the PCR comparative study, *M. genitalium* DNA were detected in 61/76 (80.3%), 52/76 (68.4%) and 74/76 (97.4%) of true-positive specimen by conventional 16S rRNA gene PCR, real-time 16S rRNA gene PCR and real-time MgPa gene PCR, respectively. Hence, real-time MgPa gene PCR is well suited for clinical diagnosis of *M. genitalium* in urogenital specimens from men and women.

The aim in paper II was to determine whether a patients’ endocervical swab specimen can be transported in first void urine (FVU) as combined specimens in detection of *Mycoplasma genitalium* by real-time PCR. The study also compared two different DNA extraction methods (manual Chelex DNA extraction and automated BioRobot M48 DNA extraction) for observation of possible PCR inhibition. Clinical specimens collected from 329 women attending a STI clinic were used in the study. A total of 100 endocervical swab specimens transported in FVU was used in the PCR inhibition analysis. *Mycoplasma genitalium* was detected in 25/329 (7.6%) women. Endocervical swab specimens transported in FVU demonstrate higher sensitivity compared to both FVU alone and specimens transported in 2-SP medium detecting 24/25 (96%), 22/25 (88%) and 17/25 (68%) of *M. genitalium* positive women, respectively. Automated BioRobot M48 DNA extraction was shown to be superior to manual Chelex extraction leaving no PCR inhibition and slightly higher DNA yield and/or better sensitivity. These two studies provide important knowledge in establishing the diagnostic level of this STI, locally, in our county and nationally.
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<tbody>
<tr>
<td>BLAST</td>
<td>Basic local alignment search tool</td>
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<tr>
<td>Bp</td>
<td>Base pairs</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for disease control and prevention, Atlanta, GA, USA</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>Ct</td>
<td>Cycle threshold</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>dNTP</td>
<td>Deoxynucleoside triphosphate</td>
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<tr>
<td>EIA</td>
<td>Enzyme immunoassay</td>
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<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
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<tr>
<td>EP</td>
<td>Ectopic pregnancy</td>
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<tr>
<td>6-FAM</td>
<td>6-Carboxyfluorescein</td>
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<tr>
<td>FRET</td>
<td>Fluorescence resonance energy transfer</td>
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<td>FVU</td>
<td>First void urine</td>
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<td>HPF</td>
<td>High power field</td>
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<tr>
<td>LAMP</td>
<td>Lipid associated membrane protein</td>
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<td>MGB</td>
<td>Minor groove binder</td>
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<td>MgPa</td>
<td>Mycoplasma genitalium adhesin protein</td>
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<td>NAAT</td>
<td>Nucleic acid amplification techniques</td>
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<td>NaCl</td>
<td>Natrium chloride</td>
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<tr>
<td>NCBI</td>
<td>National center for biotechnology information</td>
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<tr>
<td>NCNGU</td>
<td>Non-chlamydial non-gonococcal urethritis</td>
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<td>NGU</td>
<td>Non-gonococcal urethritis</td>
</tr>
<tr>
<td>NIH</td>
<td>National institute of health, Bethesda, Maryland, USA</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PID</td>
<td>Pelvic inflammatory disease</td>
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<tr>
<td>PMNL</td>
<td>Polymorph nuclear leucocytes</td>
</tr>
<tr>
<td>rMgPa</td>
<td>Recombinant Mycoplasma genitalium adhesin protein</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal RNA</td>
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<tr>
<td>SSI</td>
<td>Statens serum institute, Copenhagen, Denmark</td>
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<tr>
<td>STD</td>
<td>Sexually transmitted diseases</td>
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<tr>
<td>STI</td>
<td>Sexually transmitted infections</td>
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<tr>
<td>UHÖ</td>
<td>University Hospital Örebro</td>
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List of papers included in this thesis referred to by Roman numerals:

**Paper I**

**Paper II**
INTRODUCTION

Background
Chlamydia and gonorrhea are major causes of sexually transmitted infections (STI) in adolescents worldwide. The infections are caused by *Chlamydia trachomatis* or *Neisseria gonorrhoeae*, bacteria with clinical manifestations such as urethritis, prostatitis and epididymitis among men, and urethritis, cervicitis and upper genital tract infection (i.e. pelvic inflammatory disease) among women [14]. However, in many cases of genital tract infection, the etiology remains uncertain. *Ureaplasma urealyticum* and *Mycoplasma hominis* have been implicated as potential pathogens of the genital tract but the proportion of non-chlamydial, non-gonococcal urethritis (NCNGU) cases attributable to these pathogens is unclear [25,60]. In the continued search for pathogens, *Mycoplasma genitalium* was somewhat accidentally isolated in 1980 after prolonged incubation of specimens from men with non-gonococcal urethritis (NGU).

Biological characteristics
The genus *Mycoplasma*, belonging to the family *Mycoplasmataceae* and the class *Mollicutes* (*mollix*, soft; *cutis*, skin), comprises more than 100 different species which have been isolated from humans, animals, plants and insects. Thirteen species are at present considered as part of the human flora. Based on morphological and microbiological distinctions, the class *Mollicutes* contains six genera (*Acholeplasma*, *Anaeroplasma*, *Asteroleplasma*, *Mycoplasma*, *Sprioplasma* and *Ureaplasma*). The bacteria belonging to the class *Mollicutes* are primarily distinguished from other bacteria by their complete lack of cell wall and their minute size. In 1995, *Mycoplasma genitalium* became the second bacterium to have its complete genome fully sequenced [18]. The complete genome sequence include 580 076 base pairs (GenBank accession no. NC 000908) making it the smallest known genome of any free-living organism. Early phylogenetic analysis of rRNA genes by nucleotide cataloging revealed that mycoplasmas have evolved from gram-positive bacteria, most closely related to the clostridia and bacillus-lactobacillus cluster by degenerative evolution. More extensive analysis based upon the 5S rRNA sequences of *Mollicutes* has indicated that the initial divergence of *Mollicutes* from their clostralid ancestor probably evolved the *Acholeplasma* branch which later diverged into *Anaeroplasma* and *Sprioplasma*. Further evolutionary genome reduction of *Sprioplasmas* is suggested to have yielded the *Mycoplasma* and *Ureaplasma* lineages [63,64]. In view of this, recent mycoplasma genome projects have disclosed a remarkable lack of genes in mycoplasmas involved in biosynthetic pathways [18,24]. For instance, *M. pneumoniae* and
M. genitalium lack all genes involved in amino acid synthesis rendering them totally dependent on their host environment for supply. This might be one reason for difficulties with in vitro cultivation [60]. Mycoplasmas are primarily considered surface parasites of mucous membrane cells and their main habitat in humans are the respiratory and urogenital tracts, the eyes and sometimes joints. Mycoplasmas usually exhibit strict organ and tissue specificity with M. pneumoniae primarily found in the respiratory tract and M. genitalium preferentially found in the urogenital tract. Attachment to host target cells is mainly mediated by a tip-like organelle structure in many pathogenic mycoplasmas, which have been termed adhesins. This polar membrane extension confers a flask-shaped appearance to the M. genitalium cells. The major adhesin protein of M. genitalium (MgPa) closely resembles the main adhesin protein P1 of M. pneumoniae [10]. A prerequisite for colonization and subsequent infection of Mollicutes is adhesion to host cells. The mechanism for cell entry by mycoplasmas is still unclear, although studies with human lung fibroblasts suggest that cell adherence and entry by M. genitalium resembles cell entry by chlamydia. Mycoplasmas are known to be surface parasites but can, under certain circumstances, reside within cells that are not naturally phagocytic. The tissue damage caused by mycoplasmas at infection seems to be conveyed in part by bacterial metabolites (i.e. peroxide and superoxide radicals) causing oxidative damage to the host cell membrane. Research over the past decade has discovered genetic systems in mycoplasma cells enabling them to rapidly change their surface antigenic characteristics. This molecular mimicry allows for an adaptation to a wide range of habitats and increasing the possibility for bacteria to circumvent the host immune system. Induction of cytokines, as for many bacteria, is a recognized virulence factor of mycoplasmas mediating tissue pathology in infectious diseases [47,66].

Figure 1. Electron micrograph of M. genitalium with the specialised tip structure (arrow) [53].
History

*Mycoplasma genitalium* became the twelfth mycoplasma species known to exist and to be recovered from human origin when it was first isolated in 1980. It had been apparent to the researcher, David Taylor-Robinson, for some time, that it was unlikely that NGU in all patients could be accounted by infections with *Chlamydia trachomatis* or *Ureaplasma urealyticum*. Dark-field microscopy of fresh preparations of urethral discharges revealed motile spiral forms which he believed to be spiroplasmas. However, spiroplasmas are helical mycoplasmas present in plants and insect hosts. Taylor-Robinson thought that this might be a human counterpart.

Around this time, Joseph Tully and colleagues at the National Institute of Health (NIH) in Bethesda, Maryland, USA had developed SP4-medium for the isolation of spiroplasmas and other mycoplasmas. In 1980, Taylor-Robinson took urethral specimens from 13 men with NGU who were attending the sexually transmitted disease clinic at St. Mary’s Hospital, London, UK to Tully’s laboratory at NIH as part of collaboration. The specimens were inoculated in SP4-medium and after about 50 days of incubation at both 30°C and 37°C an acidic color change, indicating growth, could be observed in two specimens. After about eight passages of the strains in SP4-medium, the color change took place within a week. Growth on SP4 agarose medium were feasible and both strains produced, as a consequence of growth in the depth of the agar, the classical "fried-egg"-type colonies. The isolates were subjected to electron microscopy which revealed a microorganism with morphological features distinct from spiroplasmas but very similar to that of *Mycoplasma pneumoniae*. The two strains yielded were designated G-37 and M-30. Based on biological and serological properties distinct from other known mycoplasmas the mycoplasma was in 1983 named *Mycoplasma genitalium* due to its host tissue localization [53,57].

Clinical manifestations

Infections with pathogenic mycoplasmas are rarely of the fulminate type, but rather tend to follow a more chronic course. Many patients are infected by mycoplasmas without evident illness. In men, urethritis is one of the most commonly sexually transmitted infections. Many papers have been published on the role of *M. genitalium* in male NGU [7,19,25,59]. Furthermore, the highest prevalence of *M. genitalium* is detected among men with NCNGU [46]. Although asymptomatic urethritis is common, symptoms in male NCNGU include discharge and/or dysuria [16]. The role of *M. genitalium* in bacterial prostatitis and epididymitis are relatively unknown since studies of this kind in men are lacking. Nevertheless, *M. genitalium* has been found in the urethra of men with epididymitis [15] and in prostatic tissue biopsies of men with
prostatitis [39]. In women, there is a strong support for the role of *M. genitalium* in the etiology of asymptomatic and symptomatic cervicitis and urethritis [17,41,46]. Genital tract symptoms in women include intermenstrual bleeding, genital discharge or dysuria and pelvic pain. Complications from cervicitis include endometritis, pelvic inflammatory disease (PID) and adverse outcome of pregnancy and the newborn. The evidence for a role of *M. genitalium* as a cause of PID is accumulating [22,48,49]. *Mycoplasma genitalium* has been isolated in the endometrium [12] and fallopian tube [13] of women who have PID. Studies on the association of *M. genitalium* and tubal factor infertility are few, but results indicate that infections by the organism have an impact on impaired fertility in women resulting from a permanent damage of the fallopian tubes by the bacteria [21,50]. A serological study on the connection between *M. genitalium* and ectopic pregnancy (EP) showed no statistically significant association regarding EP and the presence of *M. genitalium* antibodies although a slight trend was observed in younger individuals [37]. *Mycoplasma genitalium* have been detected in extra genital sites such as synovial fluid [54,56] and conjunctival specimens [6].

**Epidemiology**

*Mycoplasma genitalium* is sexually transmissible with transmission rates similar to those of *Chlamydia trachomatis*. Prior to the advent of PCR based detection methods for *Mycoplasma genitalium* in the 1990s, few epidemiological studies have been performed on *M. genitalium* due to difficulties in culturing the organism. A meta analysis by J. S. Jensen in 2006 [27] demonstrated that a number of clinical studies have been conducted showing a prevalence of 19.3% of *M. genitalium* in men with NGU and 21.9% in men with NCNGU. The analysis also showed that the number of patients with urethritis caused by *M. genitalium* is probably smaller then the number caused by *C. trachomatis*. Jensen emphasized the need for further studies in women since the role of *M. genitalium* is less well established, particularly the relation to PID. Since then, more studies have been conducted addressing both the prevalence and the relation of *M. genitalium* to upper genital tract infections in women [22,48,49].

**Treatment**

Cell wall free organisms such as mycoplasmas are intrinsically resistant to beta-lactam antibiotics and other cell wall inhibitors. They are in general susceptible to protein synthesis inhibiting antibiotics and *in vitro* studies have showed *M. genitalium* to be susceptible to tetracyclines (doxycycline), macrolides (azithromycin) and some of the newer quinolones (moxifloxacin) [5]. Standard treatment regimens for non-gonococcal urethritis and cervicitis in STI-clinic
attendees in Scandinavia include doxycycline and/or azithromycin as primary treatment. The microbiological eradication rate of doxycycline vs. azithromycin with regard to different dosages and length of treatment have been evaluated in a small number of studies. Treatment trials from Australia in patients given azithromycin 1 g stat. show failure to eradicate *M. genitalium* in as many as 28% of men with NGU [9]. Cure rates of *M. genitalium* infection, in men and women, following azithromycin 1 g stat., are shown to be 84% [8]. In Norway, azithromycin 1 g stat. is routinely given as treatment of NGU. In a recent Norwegian study [34], a single dose of 1 g, a single dose with an extra 1 g given after one week and 1.5 g given over five days, of azithromycin, showed similar efficacy (74-79%) of microbiological cure rates when administered as a first-line treatment. The study also concludes that moxifloxacin is the best choice in cases of persistent infection after treatment failure with azithromycin. However, in the addendum of the published study, the authors’ advice doxycycline as first-line treatment of NGU since, after finishing their study, it has been shown that single dose of azithromycin may induce resistance in *M. genitalium* [31]. In a Swedish controlled clinical trial of antibiotic treatment of symptomatic *M. genitalium* infections the eradication rate after azithromycin 1 g stat. was 85% in men and 88% in women. The equivalent number following doxycycline treatment was 17% in men and 37% in women. A treatment efficacy lower than 95% is generally not considered acceptable in treatment of STI, the study showed however, that extended treatment with azithromycin in doxycycline treatment failures eradicated *M. genitalium* in 96-100% of the cases [5]. The outcome of this study has changed the recommended first-line treatment of *M. genitalium* infections in Scandinavia to azithromycin 500 mg on day 1 and 250 mg on days 2-5. Second-line treatment has not been widely studied although moxifloxacin seems to eradicate *M. genitalium* very well in patients experiencing azithromycin treatment failure. However, moxifloxacin is expensive and used improperly; the risk of developing quinolone resistance is apparent.
Diagnostic aspects

Culture

Mycoplasmas are small enough to penetrate the surface of agar medium and as a consequence of growth in the depth of the agar, the classical "fried-egg"-type colonies are seen when viewed microscopically from above [53]

![Schematic representation of a mycoplasm colony developing in, and on, agar medium.](image)

Following the initial isolation in 1980 [57], repeated attempts have been made to recover the extremely fastidious organism from clinical samples by culture techniques, but isolates have been rare and difficult to obtain. The SP4-medium developed by Joseph Tully and colleagues [58] has proved invaluable for the isolation of mycoplasmas [38]. Growth of mycoplasmas in this medium produces an acidic colour change. For propagation of *Mycoplasma genitalium* in cell culture, Vero Cells have proven useful [23,32]. However, culture methods for detection of *M. genitalium* are both laborious and time consuming and hence not used in clinical practice.
**Antibody detection**

Serology for the diagnosis of *M. genitalium* infection has not been widely used because of cross-reactivity with other mycoplasmas [40,55]. In 1997, Wang et al. [62] developed a lipid-associated membrane protein (LAMP) ELISA for detection of *M. genitalium* antibodies. LAMPs are highly antigenic, species-specific, lipid-modified proteins attached on the exterior surface of mycoplasma membranes. This method, with modification, has subsequently been used to study disease pathogenesis in women as part of an intervention trial to reduce the recurrence of STI [4]. An adaptation of the LAMP ELISA to an associated membrane protein-enzyme immunoassay (LAMP-EIA) was used to evaluate the association between *M. genitalium* antibodies and pelvic inflammatory disease and ectopic pregnancy [37]. Recently, an *M. genitalium*-specific ELISA was developed using recombinant fragments of the C-terminal part of the *Mycoplasma genitalium* adhesin protein [52]. The rMgPa ELISA was used to study *M. genitalium* antibodies in men with and without urethritis. A close-to-significant correlation between previous urethritis and antibodies to *M. genitalium* was found in addition to men with recurrent urethritis having a significantly higher IgG response than men without urethritis or acute urethritis. The study, however, used a single serum sample for serological testing (IgG) which does not discriminate between a previous, recurrent, or acute *M. genitalium* infection. Follow up serum samples are needed to establish an acute infection. Although, demonstration of an IgG titer increase in follow-up samples in men with recurrent infection might be impaired by a high antibody titer from a previous infection with *M. genitalium*. Serological methods are therefore not suitable for clinical diagnostics of an acute *M. genitalium* infection but to be used when no etiological agent can be found in a patient with invasive infection.

**Nucleic acid amplification tests**

With the development of PCR methods in the early 1990s, detection of *M. genitalium* infection became more feasible. In 1991, conventional PCR for *M. genitalium* targeting the *M. genitalium* adhesin protein gene was introduced [33]. This method was subsequently used in *M. genitalium* studies [19,43]. Conventional PCR includes an end-point analysis, usually electrophoresis of the PCR product on an agarose gel which is later stained with ethidium bromide to visualize the PCR product. Working with agarose gels is both laborious and time consuming and requires a subjective reading of the gel. Post-PCR handling also increases the risk of contaminating the environment with PCR amplicons.

In 2004, Jensen et al. [29] presented a further development of the MgPa gene PCR for application in real-time PCR. Other frequently employed PCR methods are based on the 16S rRNA
gene of *M. genitalium* which have also been used with both conventional and real-time PCR to detect *M. genitalium* infection [7,30,36]. The first real-time PCR assay for *M. genitalium* was developed by Yoshida *et al.* in 2002 [65]. The assay was based on detection of the 16S rRNA gene. The advantages of real-time PCR includes a closed format system where amplification and detection takes place in the PCR instruments requiring no POST-PCR handling of the PCR-tubes. This drastically reduces the risk of contamination. In addition, real-time PCR can be performed quantitatively and incorporate probe detection which offers additional specificity to the assay. Designing specific and sensitive assay for detection of *M. genitalium* using the 16S rRNA gene is somewhat complicated by the homology between the *M. genitalium* and *M. pneumoniae* 16S rRNA genes, and, furthermore, 16S rRNA genes have a predominance of forming secondary structures. It was concluded by Jensen [27] that diagnosis of *M. genitalium* infections in the future should be based on real-time PCR since these are highly sensitive and specific. One approach used by a few Swedish clinical microbiological laboratories is screening for *M. genitalium* with the 16S rRNA gene assay and confirming all positive results with the MgPa gene assay.

**DNA extraction**

In 2004, Jensen *et al.* [28] demonstrated that 28% of urethral swab specimens and 14% of FVU specimens contained less than 10 genome equivalents of *M. genitalium* DNA. Moreover, 20 and 13% of the two specimens’ types had less than 5 genome equivalents. Inhibitors and the probability of low DNA load in specimens emphasize a need for improved protocols for specimen preparation to increase the sensitivity in assays for clinical purpose. The growing demand for molecular diagnostics in clinical microbiology laboratories necessitates automated sample processing enabling a high extraction rate with reproducible processing over time. Automated DNA extraction methods utilizing e.g. silica coated paramagnetic beads for separation of nucleic acids will surely provide an attractive alternative to labour-intensive manual extraction methods.
Clinical specimens
The majority of clinical studies on *M. genitalium* published to date has used traditional sample specimens and transport media, e.g. 2-SP medium. In women, swab specimens from the urethra and/or the endocervix and/or first void urine (FVU) have been used and in men, swab specimens from the urethra and/or FVU are most commonly used. Although, sampling from the urethra in women, like in men, may be uncomfortable and painful. Several studies have demonstrated superior sensitivity of male FVU compared to urethral swabs and that an endocervical swab specimen should be supplemented with FVU in women in order to achieve higher sensitivity in *M. genitalium* detection [28,29,36]. Although analyzing two specimens separately from women (endocervical swab in transport medium and FVU) are not economically and practically justifiable, if the sensitivity of using FVU as transport medium for the endocervical swabs proves to be equivalent to analyzing the specimens separately.
AIMS

The aims of this thesis were to:

• Compare conventional 16S rRNA gene PCR, real-time 16S rRNA PCR and real-time MgPa gene PCR as detection methods for *Mycoplasma genitalium* (I).

• Determine the prevalence of *M. genitalium* in patients, both male and female, attending a sexually transmitted infections clinic in a rural area in Sweden (I).

• Determine whether a patient’s endocervical swab specimen can be transported in first void urine as combined specimens in *M. genitalium* detection by real-time PCR (II).

• Compare two different DNA extraction methods and observe possible PCR inhibition in the endocervical swabs specimens transported in FVU (II).
MATERIALS AND METHODS

Patients and clinical specimens
All new attendees at the STI clinic, Central Hospital, Karlstad, Sweden who were at risk of being infected with an STI due to unprotected sex with a new partner or having a sexual partner who was PCR-positive for *M. genitalium* were enrolled in the studies. In paper I, urogenital and/or first void urine specimens were collected from 381 men (range 18-82 years, median age of 27) and 298 women (range 17-55 years, median age of 25 years) during a period from April through October 2003. In paper II, urogenital and FVU specimens were collected from 329 women (range 15-65 years, median age of 24 years) between August 2004 and June 2005.

Sampling
In paper I, all men were asked to collect the first void urine for detection of *M. genitalium*. From women, an endocervical swab for detection of *M. genitalium* was collected using Rayon tipped wire shaft and placed in 1.5 ml 2-SP medium. All women were also asked to collect FVU for detection of *M. genitalium*. FVU was gathered in a 10 ml screw capped polypropylene tube. In all patients with a urine incubation time of ≤ 1 h a urethral swab specimen was collected instead of FVU using Rayon tipped wire shaft and placed in 1.5 ml 2-SP medium. In paper II, two endocervical swabs for detection of *M. genitalium* were collected using Dacron tipped plastic shaft. All women were in addition asked to collect FVU for detection of *M. genitalium*. The FVU was distributed into two 10 ml screw capped polypropylene tubes. On a two-week rotating schedule, the first endocervical swab were placed in 2-SP transport medium and the second endocervical swab were placed in one of the FVU tubes. The next week the first endocervical swab were placed in one of the FVU tubes and the second endocervical swab were placed in 2-SP transport medium.

In the PCR comparative study in paper I we used 213 specimens; 98 consecutively sampled specimens from patients enrolled in the prevalence study, 36 consecutively sampled specimens from patients with symptoms of urethritis and 79 selected specimens from patients positive for *M. genitalium* in the prevalence study by real-time MgPa gene PCR. Original specimens were sent to the Mycoplasma Laboratory at Statens Serum Institute (SSI), Copenhagen, Denmark for conventional 16S rRNA gene PCR. Chelex DNA extracts were sent to the Department of Clinical Microbiology, University Hospital Örebro (UHÖ) for real-time 16S rRNA PCR.
In the PCR inhibition analysis in paper II, one hundred MgPa gene PCR negative endocervical swab specimens transported in FVU were used. The specimens were subjected to both manual Chelex and automated DNA extraction. By adding one microliter aliquot of purified *M. genitalium* DNA per patient to the real-time MgPa gene PCR reaction mixture a comparable cycle threshold (Ct) value was created. A reference sample was created by calculating the mean Ct-value of three consecutive samples using sterile water as template. The Ct-values obtained from each patient sample was then compared to the mean Ct-value of the reference, dCt = Ct_{sample} - Ct_{reference}. A dCt-value of ≤ 3 was considered as no inhibition, i.e. less than a 10-fold decrease in analytical sensitivity. A dCt-value > 3 were considered as partial inhibition and a negative sample Ct-value were considered as total inhibition.

**DNA extraction**

The chelating resin Chelex 100 (Bio-Rad Laboratories), commonly used for extracting DNA from forensic-type samples for use with the PCR, was used for DNA isolation in papers I and II. The Chelex resin has a high affinity for polyvalent metal ions which might act as catalysts in the breakdown of DNA at high temperatures [61]. In paper II, automated DNA extraction using the BioRobot M48 (MagAttract DNA Mini Kit) [Qiagen] was used in a comparison with manual Chelex extraction to study possible PCR inhibition following the different DNA extraction methods. The MagAttract technology utilizes silica-based DNA purification by means of magnetic particles. DNA binds to the silica surface of the magnetic particles in the presence of a chaotropic salt. DNA bound to the magnetic particles is then washed followed by elution of the purified DNA in water.

For the manual Chelex DNA extraction, in papers I and II, a volume of 1800 µl from FVU and/or endocervical swab specimens transported in FVU was pelleted by centrifugation at 20 000 g for 15 min. Aliquots of swab specimen (100 µl) in 2-SP medium were mixed with 1 ml of 0.85 % NaCl prior to centrifugation. The pellet were resuspended in 300 µl of 5 % (w/v) Chelex 100 slurry (BioRad) in distilled water, vortexed for 60 s and incubated at 99 °C for 10 min. Finally, the specimens were centrifuged at 12 000 g for 5 min and a two and five microli- ter aliquot of template DNA was analysed in real-time 16S rRNA gene PCR and real-time MgPa gene PCR respectively. For conventional PCR, 100 µl of swab specimen in 2-SP me- dium was mixed with 300 µl of 20 % (w/v) Chelex 100 slurry in TE buffer, vortexed for 60 s.
and incubated at 95 °C for 10 min. After centrifugation at 20 000 g for 5 min a ten microliter aliquot of template DNA was analysed in conventional 16S rRNA gene PCR.

For the automated DNA extraction, in paper II, a volume of 1800 µl from endocervical swab specimens transported in FVU was pelleted by centrifugation at 20 000 g for 15 min. The pellet were resuspended in 200 µl phosphate buffered saline (PBS) and vortexed thoroughly. The BioRobot M48 (MagAttract DNA Mini Kit [Qiagen]; with 200 µl sample input and 100 µl sample output) was used according to the manufacturer’s instructions. A five microliter aliquot of template DNA was analysed in real-time MgPa gene PCR.

**Diagnostic tests**

**PCR**

In paper I, conventional and real-time 16S rRNA gene PCR used primers MG16-45F (5´- TAC ATG CAA GTC GAT CGG AAG TAG C -´3) and MG16-447R (5´- AAA CTC CAG CCA TTG CCT GCT AG -´3) to amplify a 427 bp fragment of the 16S rRNA gene of *M. genitalium* (GenBank accession no. X77334). In the conventional PCR assay, a Perkin Elmer 9600 Thermal Cycler instrument was used for amplification and the amplicons were visualized after electrophoresis on 2 % agarose gels containing 1 µg/ml of ethidium bromide and examined by UV transillumination. In the real-time PCR assay, a LightCycler instrument was used for amplification and the PCR products was detected using fluorescence resonance energy transfer (FRET) probes Mg16S-137 (LC-red 640 AAT TCA TGC GAA CTA AAG TTC TTA TGC GGT ATT AGC T – phosphate) and Mg16S-169 (AAT AAC GAA CCC TTG CAG GTC CTT TCA ACT T –fluorescein). In papers I-II, real-time MgPa gene PCR was carried out in a SmartCycler instrument using the forward primer MgPa-355F (5´- GAG AAA TAC CTT GAT GGT CAG CAA -´3), reverse primer MgPa-432R (5´- GTT AAT ATC ATA TAA AGC TCT ACC GTT GTT ATC -´3) and MgPa-380 TaqmanMGB probe (FAM-ACT TTG CAA TCA GAA GGT -MGB) amplifying a 78 bp fragment of the MgPa gene sequence (GenBank accession no. M31431).

**DNA Sequencing**

Purified PCR products were sequenced with ABI® BigDye ™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) using the MgPa gene real-time PCR primers. Sequencing reactions were purified using DyeEx ™ 2.0 Spin Kit (Qiagen) before separation using ABI PRISM® 310 Genetic Analyzer (Applied Biosystems). The nucleotide sequences
were analyzed using CromaPro version 1.33 software and compared to sequence databases at the National Centre for Biotechnology Information (NCBI), with the basic local alignment search tool (BLAST) at [http://www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/).

**Statistical analysis**
All statistical analysis, McNemar's test to compare paired proportions using the two-tailed \( P \) value and 95 % confidence intervals (CI), was performed with GraphPad Quickcalcs ([http://www.graphpad.com/quickcalcs/index.cfm](http://www.graphpad.com/quickcalcs/index.cfm)) [GraphPad Software Inc.].
RESULTS AND DISCUSSION

Prevalence and PCR comparative study in the detection of *Mycoplasma genitalium* (I)
The purpose of paper I was to compare different PCR assays for detection of *M. genitalium* in urogenital specimens from men and women and to determine the prevalence of *M. genitalium* in patients, both men and women, attending an STI clinic in the study catchments area. The results from this study will aid in establishing the future diagnostic level of this STI in our county and also nationally.

**Prevalence**

To date, there are only a few studies addressing the prevalence of *Mycoplasma genitalium* infection in both men and women from the same catchments area in Sweden. In order to determine the prevalence of *M. genitalium* in paper I, urogenital specimens from patients, both male and female, attending an STI clinic in our catchments area were collected. *Mycoplasma genitalium* was detected in 27/381 (7.1 %) (95 % CI = 4.72 - 10.14 %) men and in 23/298 (7.7 %) (95 % CI = 4.96 - 11.36 %) women by real-time MgPa gene PCR (Tables 1 and 2). Clinical information from the 27 *M. genitalium* PCR positive men showed a 59 % concordance between a positive PCR result and symptoms of urethritis and/or a positive urethral smear indicating urethritis. Clinical information from the 23 *M. genitalium* PCR positive women showed a 30 % concordance between a positive PCR result and symptoms of cervicitis and/or a positive wet smear indicating cervicitis. This is a lower proportion than previous reports for men but in congruence with other reports for women [16,17]. The lesser percentage of symptom concordance for men could be attributed to the fact that a less sensitive plastic loop or a swab was used instead of a blunt curette in sampling.

**Table 1.** Distribution of *M. genitalium* PCR positive results and specimen sets from 381 men included in the prevalence study

<table>
<thead>
<tr>
<th>Specimen type or combination</th>
<th>Urethral swab (n = 15)</th>
<th>First Void Urine (n = 361)</th>
<th>Urethral swab + First Void Urine (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. genitalium</em> positive</td>
<td>0</td>
<td>27 (7.5 %)</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2. Distribution of *M. genitalium* PCR positive results among 298 women included in the prevalence study

<table>
<thead>
<tr>
<th>Specimen type or combination</th>
<th>Cervical swab ( (n = 30) )</th>
<th>First Void Urine ( (n = 4) )</th>
<th>Cervical swab + Urethral swab ( (n = 9) )</th>
<th>Cervical swab + First Void Urine ( (n = 255) )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. genitalium</em> positive</td>
<td>2 (6.7 %)</td>
<td>0</td>
<td>2 (22.2 %)</td>
<td>19 (7.5 %)</td>
</tr>
</tbody>
</table>

Studies on *M. genitalium* in different patient populations have previously been conducted in Sweden. In 2000, Bjornelius *et al.* [7] correlated NGU and NCNGU in Swedish male STI patients to the presence of *M. genitalium* in urethral swabs showing an occurrence of 26% and 36% by *M. genitalium* in patients with NGU and with NCNGU respectively, compared to 10% in control patients without urethritis. This is in congruence with previous international reports for patients with NGU and NCNGU but a somewhat higher percentage of *M. genitalium* infected control patients without urethritis compared to previous reports (0.8-8.5 %) [3,26]. Also in 2000, Johannisson *et al.* [35] evaluated the prevalence and the relation of *M. genitalium* to the number of lifetime sexual partners in patients visiting STI clinics in western Sweden. The study showed considerably lower numbers of infected patients compared to the study by Bjornelius *et al.* Seven percent of the examined men, 14% of men with urethritis and 1% of the male control patients without urethritis were infected with *M. genitalium*. Other Swedish studies have showed prevalence in concordance with our results [2,16,17] while Mellenius *et al.* [44] presented slightly lower numbers of prevalence compared to our study, 4.1% in men and 3.8% among women.

In a study from USA by Gaydos *et al.* [20], the prevalence of *Mycoplasma genitalium* compared to *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* in male STI attendees with and without urethritis have been investigated. The study demonstrated an overall prevalence for *M. genitalium*, *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis* of 15.2%, 20.3%, 12.8% and 3.4%, respectively. The prevalence of each organism in men with urethritis was 22.4%, 32.7%, 24.2% and 5.2% compared to 7.3%, 6.6%, 0% and 1.5% in men without urethritis for *M. genitalium*, *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis*, respectively.

Individuals attending STI clinics have been the group most thoroughly studied and little is known about the infection in the general population. The few studies that have been conducted in the general population show a low prevalence of *M. genitalium* in men (1.1% prevalence)
and in women (0 - 2.3 % prevalence). These studies have been conducted in Denmark and Vietnam [3,45]. On the basis of the low prevalence in the general population, a widespread screening for *M. genitalium* seems unwarranted at present.

**PCR comparative study**

A total of 213 specimens were used in the PCR comparative study. None of the assays evaluated were considered a “gold standard” assay, hence the relation of agreement in analysis results between the assays was calculated. The real-time MgPa gene PCR assay established a 92 % and 89.7 % agreement in comparison of analysis results obtained with the conventional 16S rRNA gene PCR and real-time 16S rRNA gene PCR assays respectively. A 93 % agreement was demonstrated in analysis results with comparison between real-time 16S rRNA gene PCR and conventional 16S rRNA gene PCR assays. Seventy-six specimens were considered as true-positive specimens defined as, either a specimen positive in any two PCR assays, or a specimens PCR product verified by DNA sequencing. Forty-nine specimens were positive in all three assays (Figure 3).

![Figure 3](image_url)

**Figure 3.** Distribution of 213 specimens (76 positive and 137 negative) in a comparison of real-time 16S rRNA gene PCR, conventional 16S rRNA gene PCR and real-time MgPa gene PCR assays for detection of *M. genitalium*. Positive specimens are indicated within the circles.

† Four of these specimens came from women with another *M. genitalium* true-positive sample  
‡ These two specimens came from women with another *M. genitalium* true-positive sample  
¥ Specimens re-tested with real-time MgPa gene PCR and the PCR products were verified by DNA sequencing

Twelve specimens were found positive for *M. genitalium* only by real-time MgPa gene PCR. Two specimens were found positive for *M. genitalium* only by conventional 16S rRNA gene PCR. These specimens were re-tested with real-time MgPa gene PCR and the PCR products were verified by DNA sequencing (data not shown). No re-tested specimens could be subjected to a new DNA extraction due to insufficient specimen material left after previous DNA
extractions. Real-time 16S rRNA gene PCR has previously been demonstrated to have a lower sensitivity for detection of *M. genitalium* in urogenital specimens from men compared to conventional 16S rRNA gene PCR. Jurstrand *et al.* [36] showed 72.2% sensitivity and 99.7% specificity for real-time PCR compared to conventional PCR for detection of *M. genitalium*. Furthermore, real-time MgPa gene PCR has recently been compared to a newly developed real-time PCR method, real-time gap-gene PCR, and to conventional 16S rRNA gene PCR as detection methods for *M. genitalium*, but only in urethral swab specimens from men. Real-time MgPa gene PCR proved to be a very sensitive method detecting low copy numbers of *M. genitalium* DNA (range 0.03 – 2.65 copies/µl) not detected by the real-time gap-gene PCR [51]. Conventional 16S rRNA gene PCR also detected specimens with low copy numbers (< 3 copies/µl) not detected by the real-time gap-gene PCR who was performed on a LightCycler instrument allowing for 2 µl of template DNA to be analyzed. Jensen *et al.* [28] demonstrated that 28% of urethral swab specimens and 14% of FVU specimens contained less then 10 genome equivalents of *M. genitalium* DNA. Clearly, a low load of *M. genitalium* DNA in clinical specimens underlines the need for highly sensitive assays and if possible, a larger volume of template DNA to be analysed. In our PCR comparative study the real-time 16S rRNA gene PCR was performed on a LightCycler allowing only 2 µl of template DNA to be analyzed while the real-time MgPa gene PCR was performed on a SmartCycler using 5 µl of template DNA. This could explain the lower sensitivity for the real-time 16S rRNA gene assay. However, the conventional 16S rRNA gene PCR had the advantage of being performed with a total volume of 100 µl allowing for 10 µl of template DNA to be analyzed. The conventional assay increased the sensitivity of the 16S rRNA gene assay somewhat but evidently, using the MgPa gene as target provides a more sensitive PCR method for detection of *M. genitalium*.

In paper I, specific transport media (2-SP medium) were used to transport swab specimen, predominantly from women. Transportation of endocervical swab specimens in the patients FVU, which has previously been shown favourable for detection of *C. trachomatis* [1], have never been demonstrated for *M. genitalium*. In paper I, as well as in many other clinical studies to date, a crude Chelex extraction of DNA as sample preparation method is used. There is a need for improved protocols for sample transportation and preparation to increase the sensitivity in assays for clinical purpose and those are the reasons for the study presented in paper II.
Comparative study on DNA extraction methods in combined specimens for detection of *Mycoplasma genitalium* by real-time PCR (II)

The purpose of paper II was to determine whether a patient’s endocervical swab specimen can be transported in first void urine as combined specimens. Analyzing two separate specimens from women is not cost-effective and efficient if the sensitivity of combining the FVU with the endocervical swab is equivalent to analyzing the specimens separately. The logistics in handling a specific transport medium (e.g. 2-SP medium) is complex with limited shelf-life and the cost of producing the medium. Also, PCR inhibitors and the probability of low DNA load in specimens necessitate improved protocols for specimen preparation to increase the sensitivity in assays for clinical purpose. The results from this study will aid in establishing the future diagnostic level of this STI in our county and also nationally.

**Combined specimens**

All 329 women provided three specimens; one endocervical swab specimen transported in 2-SP medium, one endocervical swab specimen transported in FVU and also a solely FVU specimen. A specimen was considered as true positive if at least one of the three specimens in a patient’s set was positive for *M. genitalium* by real-time MgPa gene PCR. *Mycoplasma genitalium* was detected in 25/329 (7.6 %) (95 % CI = 4.98 - 11.01 %) women by real-time MgPa gene PCR. The endocervical swab specimens transported in 2-SP medium and transported in FVU were found positive for *M. genitalium* in 17/25 (68 %) (95 % CI = 46.50 - 85.05 %) and 24/25 (96 %) (95 % CI = 76.50 - 99.90 %) of women, respectively (Figure 4). Two specimens were found positive for *M. genitalium* only in the endocervical swab specimens transported in FVU. The FVU specimens alone were found positive for *M. genitalium* in 22/25 (88 %) (95 % CI = 68.78 - 97.45 %) women.
DNA extraction and inhibition analysis

A total of 329 endocervical swab specimens transported in FVU were used in the DNA extraction comparative study. *Mycoplasma genitalium* DNA was detected in 24/329 (7.3 %) (95 % CI = 4.73 - 10.66 %) and 28/329 (8.5 %) (95 % CI = 5.73 - 12.07 %) of endocervical swab specimens transported in FVU subjected to manual Chelex extraction and automated BioRobot M48 extraction, respectively. Four specimens were found positive for *M. genitalium* only by automated BioRobot M48 extraction. All four specimens were able to be re-tested and found positive. One of these specimens came from a woman with other *M. genitalium* positive samples.

One hundred PCR-negative endocervical swab specimens transported in FVU were used in the PCR inhibition analysis. Partial PCR inhibition was detected in 6 % of samples by means of the manual Chelex extraction method (Figure 5) whereas no inhibition was detected with the automated BioRobot M48 extraction (Figure 6).
**Figure 5.** PCR inhibition analysis of 100 endocervical swab specimens transported in FVU subjected to manual Chelex DNA extraction including dCt distribution as determined by real-time MgPa gene PCR. A dCt-value > 3 were considered as partial inhibition (red line). dCt = Delta cycle threshold (C_{sample} - C_{reference}).

**Figure 6.** PCR inhibition analysis of 100 endocervical swab specimens transported in FVU subjected to automated MagAttract DNA extraction including dCt distribution as determined by real-time MgPa gene PCR. A dCt-value > 3 were considered as partial inhibition (red line). dCt = Delta cycle threshold (C_{sample} - C_{reference}).
Traditional sample specimens and transport media (e.g. 2-SP medium) are widely used in clinical studies on *M. genitalium*. In a recent study by Jensen *et al.* [28], FVU specimens were shown to detect significantly more (88 %) of *M. genitalium* infections than the urethral (57 %) and endocervical (71 %) swab specimens of infected women. However, if the FVU would be supplemented with an endocervical swab specimen the sensitivity of *M. genitalium* detection could be improved to 96 %. The need of supplementing the endocervical swab specimen with FVU to increase the possibility of detecting *M. genitalium* infection has also been illustrated by Jurstrand *et al.* [36] who showed that *M. genitalium* DNA was detected in only one of the two specimens in 50 and 31 % of *M. genitalium*-infected women by real-time LightCycler PCR and conventional PCR, respectively.

The main purpose of the present study was to determine if women’s endocervical swab specimens can be transported in FVU for detection of *M. genitalium* by real-time MgPa gene PCR. It was shown to be superior to the endocervical swab specimens transported in 2-SP medium (*P* = 0.05, statistically significant difference). Solely FVU specimens were found somewhat less sensitive than endocervical swab specimens transported in FVU (*P* = 0.62, not statistically significant difference). Although few positive patients, the results indicates that pooling a cervical swab with FVU has several advantages in the diagnosis of *M. genitalium* infection for sensitivity of diagnostic test, economy and eliminating logistic distribution of a specific transport medium.

In our DNA extraction comparison of manual Chelex extraction vs. automated DNA extraction, four endocervical swab specimens transported in FVU were found positive for *M. genitalium* by real-time MgPa gene PCR subjected to automated BioRobot M48 extraction, in comparison to manual Chelex extraction, indicating, although few positive specimens, a higher sensitivity in the automated DNA extraction method (*P* = 0.13, not statistically significant difference). In the present study, 200 µl of endocervical swab specimen transported in 2-SP medium was used for manual Chelex DNA extraction in comparison to 1800 µl of endocervical swab specimen transported in FVU and solely FVU specimens. This could partly explain the lower sensitivity for the endocervical swab specimen transported in 2-SP medium.

We also performed a PCR inhibition analysis of the two different DNA extraction methods to observe possible PCR inhibition in the endocervical swab specimens transported in FVU. We
demonstrated a slightly higher DNA yield and/or better sensitivity using automated BioRobot M48 extraction in terms of PCR mean dCt-values (mean dCt -2.38, data not shown) in contrast to manual Chelex extraction where partial inhibition was observed in 6 % of samples. No inhibition was detected with automated BioRobot M48 extraction, using the MagAttract DNA Kit. Other studies have found inhibitory activities when analysing *M. genitalium* by PCR, most of which have used the crude Chelex extraction method [11,28,29,36]. Inhibitors and the probability of low DNA load in specimens emphasize a need for improved protocols for specimen preparation to increase the sensitivity in assays for clinical purpose.

Future aspects of *Mycoplasma genitalium* diagnosis predominantly include gathering further evidence of the causative role of *M. genitalium* in upper genital tract infection and the potential of reproductive sequelae. This is somewhat hampered by the lack of a commercially available test for *M. genitalium* leaving testing of this organism mainly to research laboratories using in-house PCR assays. We seem to be, as Lisa E. Manhart wrote in an editorial in sexually transmitted diseases in October 2009 [42], caught in a catch-22-diagnostic test where manufacturers are waiting for definitive evidence that *M. genitalium* causes upper reproductive tract sequelae and the inclusion *M. genitalium* in the CDC STD treatment guidelines before bringing a test to market. Clinical decision-makers are waiting for the availability of a commercial diagnostic test before calling for widespread testing for *M. genitalium*, a bacterium often more prevalent than the usual suspects’ *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in most studies conducted on the matter.
CONCLUSIONS

- Real-time MgPa gene PCR demonstrate higher sensitivity compared to conventional 16S rRNA gene PCR and considerably increase sensitivity compared to real-time 16S rRNA gene PCR for detection of *M. genitalium* DNA. It has a number of advantages over conventional PCR since it is a closed format, decreasing the contamination risk, less labour-intense and the use of probe increases specificity. This method is well suited for clinical diagnostics of *M. genitalium* in urogenital specimens from men and women (I).

- The prevalence (7.1 % in men and 7.7 % in women) of *Mycoplasma genitalium* in patients attending an STI clinic in the study catchments area is in concordance with other Swedish studies (I).

- Endocervical swab specimens transported in FVU demonstrate higher sensitivity compared to solely FVU and considerably increased sensitivity compared to endocervical swab specimens transported in 2-SP medium for detection of *M. genitalium* DNA by real-time MgPa gene PCR. Endocervical swab specimens transported in the patients FVU will save the cost of the 2-SP medium, reduce the analytical cost as two specimens becomes one and relieve logistic difficulties in distributing the 2-SP medium out to clinics (II).

- Automated BioRobot M48 extraction using the MagAttract DNA Kit was shown to be superior to a crude manual Chelex extraction leaving no PCR inhibition and slightly higher DNA yield and/or better sensitivity (II).
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REFERENCES


Chlamydia and gonorrhea are major causes of sexually transmitted infections (STI) in adolescents worldwide. The infections are caused by Chlamydia trachomatis or Neisseria gonorrhoeae, bacteria with clinical manifestations such as urethritis, prostatitis and epididymitis among men, and urethritis, cervicitis and upper genital tract infection (i.e. pelvic inflammatory disease) among women. However, in many cases of genital tract infection, the etiology remains uncertain. In light of this, Mycoplasma genitalium was somewhat accidentally isolated in 1980 after prolonged incubation of urogenital specimens from men with non-gonococcal urethritis. Following the initial isolation in 1980, repeated attempts have been made to recover this extremely fastidious organism from clinical samples by culture techniques, but isolates have been rare and difficult to obtain. With the development of PCR methods in the early 1990s, detection of M. genitalium infection became more feasible.

The aim in paper I was to compare three different PCR assays (conventional and real-time 16S rRNA gene PCR as well as real-time Mycoplasma genitalium adhesin protein (MgPa) gene PCR) for detection of M. genitalium. The study also determined the prevalence of M. genitalium. Clinical specimens collected from STI attendees, 381 men and 298 women, were used to determine the prevalence of M. genitalium and 213 of these specimens were used in the PCR comparative study. The prevalence of M. genitalium infection in men and women was 27/381 (7.1 %) and 23/298 (7.7 %) respectively. In the PCR comparative study, M. genitalium DNA were detected in 61/76 (80.3 %), 52/76 (68.4 %) and 74/76 (97.4 %) of true-positive specimen by conventional 16S rRNA gene PCR, real-time 16S rRNA gene PCR and real-time MgPa gene PCR, respectively. Hence, real-time MgPa gene PCR is well suited for clinical diagnosis of M. genitalium in urogenital specimens from men and women.

The aim in paper II was to determine whether a patients’ endocervical swab specimen can be transported in first void urine (FVU) as combined specimens in detection of Mycoplasma genitalium by real-time PCR. The study also compared two different DNA extraction methods (manual Chelex DNA extraction and automated BioRobot M48 DNA extraction) for observation of possible PCR inhibition. Clinical specimens collected from 329 women attending a STI clinic were used in the study. A total of 100 endocervical swab specimens transported in FVU was used in the PCR inhibition analysis. Mycoplasma genitalium was detected in 25/329 (7.6 %) women. Endocervical swab specimens transported in FVU demonstrate higher sensitivity compared to both FVU alone and specimens transported in 2-SP medium detecting 24/25 (96 %), 22/25 (88 %) and 17/25 (68 %) of M. genitalium positive women, respectively. Automated BioRobot M48 DNA extraction was shown to be superior to manual Chelex extraction leaving no PCR inhibition and slightly higher DNA yield and/or better sensitivity.

These two studies provide important knowledge in establishing the diagnostic level of this STI, locally, in our county and nationally.