Endocervical swabs transported in first void urine as combined specimens in the detection of *Mycoplasma genitalium* by real-time PCR

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The aim of this study was to determine whether a patient’s endocervical swab specimen can be transported in first void urine (FVU) as combined specimens for the detection of *Mycoplasma genitalium* by real-time PCR. The study also compared two different DNA extraction methods for observation of possible PCR inhibition. Three specimens, one endocervical swab specimen transported in 2-SP medium, one endocervical swab specimen transported in FVU and a FVU specimen, were collected from 329 women. All sample types underwent manual DNA extraction whereas in the DNA extraction study, 329 endocervical swab specimens transported in FVU were subjected to both manual Chelex and automated BioRobot M48 DNA extraction. A total of 100 endocervical swab specimens transported in FVU from patients PCR-negative for *M. genitalium* in the study were used in the PCR inhibition analysis. *M. genitalium* was detected in 25/329 (7.6 %) women. The endocervical swab specimens transported in 2-SP medium and transported in FVU were positive for *M. genitalium* in 17/25 (68 %) and 24/25 (96 %) women, respectively. The FVU specimens alone were positive for *M. genitalium* in 22/25 (88 %) women. In the DNA extraction study, *M. genitalium* DNA was detected in 24/329 (7.3 %) and 28/329 (8.5 %) of endocervical swab specimens transported in FVU subjected to manual Chelex extraction and automated BioRobot M48 extraction, respectively. Partial PCR inhibition was detected in 6 % of samples subjected to manual Chelex extraction whereas no inhibition was detected with the automated BioRobot M48 extraction. Thus endocervical swab specimens transported in FVU demonstrate higher sensitivity than FVU specimens only and have considerably increased sensitivity compared with endocervical swab specimens transported in 2-SP medium for detection of *M. genitalium* DNA. Moreover, automated BioRobot M48 extraction was shown to be superior to a crude manual Chelex extraction, leaving no PCR inhibition and giving a slightly higher DNA yield and/or better sensitivity.

INTRODUCTION

The well-established pathogens *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are known to cause upper genital tract infections (i.e. epididymitis in men and pelvic inflammatory disease in women) and are often associated with urethritis in men and cervicitis in women (Falk et al., 2004, 2005). In many patients with symptomatic non-chlamydial, non-gonococcal urethritis (NCNGU), the aetiology remains unclear. *Mycoplasma genitalium* has, since its first isolation in 1980 (Tully et al., 1981), been indicated as having a causative role in NCNGU in men and cervicitis in women (Deguchi & Maeda, 2002; Jensen, 2004; Taylor-Robinson, 2002; Uusku¨la & Kohl, 2002). *M. genitalium* infection has also been associated with pelvic inflammatory disease but the exact role has not yet been determined (Cohen et al., 2002). 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. With the development of PCR methods in the early 1990s, detection of *M. genitalium* infection became more feasible. Conventional sample specimens [urethra, endocervix and/or first void urine (FVU)] and transport
media (e.g. 2-SP medium containing sucrose–phosphate buffer with fetal calf serum and antibiotics) have been used in most clinical studies of *M. genitalium* published thus far. However, sampling from the urethra in women, like in men, may be uncomfortable and painful. Several studies have demonstrated the 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 (Jensen et al., 2004a, b; Jurstrand et al., 2005). Analysing two specimens separately from women (endocervical swab in transport medium and FVU) would not be economically and practically justifiable if the sensitivity of pooling the FVU with the endocervical swab proves to be equivalent to analysing the specimens separately. The aim of this study was to determine whether a patient’s endocervical swab specimen can be transported in FVU as combined specimens in *M. genitalium* detection by real-time PCR. In addition, we also wanted to compare two different DNA extraction methods to observe possible PCR inhibition in the endocervical swab specimens transported in FVU.

**METHODS**

**Patients and clinical specimens.** From August 2004 to June 2005, specimens were obtained from 329 women (15–65 years of age, median 24 years) attending the STI clinic at the Central Hospital Karlstad, Sweden. All new attendees who were at risk for being infected with a sexually transmitted infection, due to unprotected sex with a new partner or having a sexual partner who was PCR-positive for *M. genitalium*, were enrolled in the study after providing informed consent. Two endocervical swabs for detection of *M. genitalium* were collected using a Dacron-tipped plastic shaft Copan 159 C swab. Following the clinical examination, all women were asked to collect a specimen only.

**Specimens**

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**DNA extraction**

**Manual extraction.** A volume of 1800 μl from FVU and endocervical swab specimens transported in FVU was pelleted by centrifugation at 20 000 g for 15 min. The pellet was resuspended in 200 μl PBS and vortexed thoroughly. The BioRobot M48 [MagAttract DNA Mini kit (Qiagen); 200 μl sample input, 100 μl output] was used according to the manufacturer’s instructions. A 5 μl aliquot of template DNA was analysed in real-time MgPa gene PCR.

**Automated extraction.** 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 was resuspended in 200 μl PBS and vortexed thoroughly. The BioRobot M48 [MagAttract DNA Mini kit (Qiagen); 200 μl sample input, 100 μl output] was used according to the manufacturer’s instructions. A 5 μl aliquot of template DNA was analysed in real-time MgPa gene PCR.

**RESULTS**

**Specimens**

All 329 women provided three specimens: one endocervical swab specimen transported in FVU and also a FVU specimen only. *M. genitalium* was detected in 25/329 (7.6%) women by real-time MgPa gene PCR. The endocervical swab specimens transported in 2-SP medium and transported in FVU were positive for *M. genitalium* in 17/25 (68%) and 24/25 (96%) women, respectively (Fig. 1). Two specimens were positive for *M. genitalium* only in the endocervical swab specimens transported in FVU. Both specimens were able to be retested and were found to be repetitively positive. The FVU specimens alone were positive for *M. genitalium* in 22/25 (88%) women.
DNA extraction and inhibition analysis

A total of 329 endocervical swab specimens transported in FVU were used in the DNA extraction comparative study. M. genitalium DNA was detected in 24/329 (7.3 %) and 28/329 (8.5 %) endocervical swab specimens transported in FVU subjected to manual Chelex extraction and automated BioRobot M48 extraction, respectively. Four specimens were found to be positive for M. genitalium only by automated BioRobot M48 extraction. All four specimens were able to be retested and found to be 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 subjected to manual Chelex extraction whereas no inhibition was detected with the automated BioRobot M48 extraction (Table 1).

DISCUSSION

To the best of our knowledge, this is the first comparison between endocervical swab specimens transported in 2-SP medium, endocervical swab specimens transported in FVU and FVU alone for detection of M. genitalium infection in women. It is also the first comparison of manual Chelex extraction and automated BioRobot M48 extraction using the MagAttract DNA kit for detection of M. genitalium. The majority of clinical studies on M. genitalium published to date have 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 FVU have been used and, in men, swab specimens from the urethra and/or FVU are most commonly used. In a recent study by Jensen et al. (2004a), significantly more (88 %) M. genitalium infections were detected in FVU specimens than in urethral (57 %) and endocervical (71 %) swab specimens of infected women. However, if the FVU was supplemented with an endocervical swab specimen the sensitivity of M. genitalium detection could be improved to 96 %. In 2005, Jurstrand et al. (2005) illustrated the need to analyse both endocervical swabs and FVU since 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. However, analysing 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 that when the specimens are analysed separately. The main purpose of the present study was to determine whether women's endocervical swab specimens can be transported in FVU for detection of M. genitalium by real-time MgPa gene PCR. This method was shown to be superior to transporting the endocervical swab specimens in 2-SP medium. FVU specimens only were somewhat less sensitive

Table 1. Comparison of PCR inhibition in 100 endocervical swab specimens transported in FVU subjected to manual Chelex and automated MagAttract DNA extraction, including dCt distribution as determined by real-time MgPa gene PCR.

dCt, Delta cycle threshold (C_{t, sample} − C_{t, reference}).

<table>
<thead>
<tr>
<th>Chelex extraction</th>
<th>MagAttract DNA extraction</th>
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<tr>
<td>No inhibition</td>
<td>94 %</td>
</tr>
<tr>
<td>Partial inhibition</td>
<td>&lt;1 %</td>
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<tr>
<td>Total inhibition</td>
<td>&lt;1 %</td>
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<tr>
<td>dCt mean</td>
<td>0.99</td>
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<tr>
<td>dCt max</td>
<td>9.27</td>
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<tr>
<td>dCt min</td>
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Fig. 1. Distribution of 329 specimen sets from women as determined by real-time MgPa gene PCR. M. genitalium-positive specimens are indicated within the circles.
than endocervical swab specimens transported in FVU. Although there were few positive patients, the results indicate that pooling a cervical swab with FVU has several advantages in the diagnosis of *M. genitalium* infection, such as sensitivity of the diagnostic test, economy, and comfort for the woman. Transportation of the endocervical swab specimen in the patient’s FVU has previously been shown favourable for detection of *C. trachomatis*, with a sensitivity of 97.9 % in pooled specimens of FVU and endocervical swabs compared to 93.3 % in FVU alone (Airell et al., 2000). Data were in agreement with the present study for *M. genitalium* detection.

Four endocervical swab specimens transported in FVU were found positive for *M. genitalium* by real-time MgPa gene PCR when subjected to automated BioRobot M48 extraction, but not when manual Chelex extraction was used, indicating a higher sensitivity for the automated extraction, but not when manual Chelex extraction was used, indicating a higher sensitivity for the automated extraction, but not when manual Chelex extraction was used, indicating a higher sensitivity for the automated DNA extraction method. 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 FVU specimens only. This could partly explain the lower sensitivity for the endocervical swab specimen transported in 2-SP medium.

In the present PCR inhibition analysis, two different DNA extraction methods were compared to observe possible PCR inhibition in the endocervical swab specimens transported in FVU. We demonstrated a slightly higher DNA yield and/or better sensitivity in terms of PCR mean dCt values (mean dCt = −2.38, data not shown) using automated BioRobot M48 extraction compared 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 (Jensen et al., 2004a, b; Jurstrand et al., 2005). Jensen et al. (2004a) 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 specimen 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 purposes.

In conclusion, endocervical swab specimens transported in FVU demonstrate higher sensitivity than FVU specimens only 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. Moreover, 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 giving a slightly higher DNA yield and/or better sensitivity. Endocervical swab specimens transported in patients’ FVU will save the cost of the 2-SP medium, reduce the analytical cost as two specimens become one and relieve logistic difficulties in distributing the 2-SP medium out to clinics.

**ACKNOWLEDGEMENTS**

We are grateful for the excellent work of the staff at the outpatient STI clinic, Karlstad Central Hospital, during the study. This work was partly supported by grants from the Värmland County Hospital Foundation.

**REFERENCES**


