

# Detection of potential sexually transmitted bacterial pathogens by molecular methods

**Keywords:** *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*

## Introduction

In recent year many studies have reported the increased importance of sexually transmitted organisms as cause of urogenital diseases and especially their potential to develop infertility.<sup>1,2</sup> In particular, *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Ureaplasma urealyticum* are among the most prevalent pathogens. Therefore, an accurate diagnosis of their infections and administration of effective therapy is important to prevent their complications in future.

The following is a short description of these organisms.

### *C. trachomatis*

This bacterium is the most common cause of sexually transmitted infections (STIs) in developed countries. It is an obligate intracellular bacterium, which has a unique biphasic developmental cycle.<sup>3</sup> It causes often a diverse of urogenital diseases such as nonspecific urethritis, epididymitis and proctitis in men, whereas causes cervicitis, urethritis and pelvic inflammatory disease (PID) in women and inclusion conjunctivitis and neonatal pneumonia. A special L-serotype of *C. trachomatis* causes Lymphogranuloma Venereum (LGV), mostly in patients co-infected with HIV.<sup>4</sup> In addition, between 50 to 70% of all Chlamydia genital infections in men and women may be asymptomatic and remain undiagnosed and untreated.<sup>3</sup> In particular, Chlamydia infection in women may lead to late complications such as PID, ectopic pregnancy or tubal factor infertility.<sup>5,6</sup>

**Laboratory diagnosis:** Cell culture of *C. trachomatis* has fast 100% specificity, but it is not practical for routine use, because lack of high sensitivity associated with technical complexity in transport, storage and collection of adequate specimens.<sup>3</sup> A diagnosis is best made by using nucleic acid amplification tests, because such tests show a good specificity as well as being highly sensitive and do not require invasive procedures for specimen collection.<sup>7</sup> DNA probing was the first molecular DNA test for *C. trachomatis*, and was largely used before the advent of nucleic acid amplification test.<sup>3</sup> One commercially available probe test (PACE 2, Gen-Probe Inc, USA) uses DNA-RNA hybridization in an effort to increase sensitivity by detecting chlamydial RNA. Available data suggest that this probe test is relatively specific and provides sensitivity similar to that of the better antigen detection and cell culture methods.<sup>3,7</sup>

Nucleic Acid Amplification Tests (NAATs) are becoming the tests of choice for the diagnosis of *C. trachomatis* genital infections, because of their high sensitivity and specificity, and their possible use for a large range of sample types, including vulvovaginal swabs and first voided urine (FVU).<sup>3</sup> Several commercial NAATs are available, and make use of different technologies: Polymerase chain reaction PCR and real-time PCR (Roche Diagnostics, Abbott, IL, USA), ligase chain reaction LCx assay (Abbott Laboratories, USA), strand displacement amplification (Becton Dickinson, NJ, USA); transcription-mediated amplification (Gen Probe); and nucleic acid

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sequence-based amplification (bioMerieux, Nancy L'Etoile, France).<sup>3,7</sup> Only approved specimens as outlined in each package insert should be tested in these assays.<sup>7</sup> The major targets for amplification-based tests are generally multiple copy genes, which are carried by the cryptic plasmid of *C. trachomatis*, or gene products such as rRNAs.<sup>3,7</sup> All of the molecular assays appear to be highly specific if problems with cross contamination of reactions are avoided. Clinical evaluations of these amplification methods have demonstrated higher sensitivity than culture and the other non-culture methods (microscopy, immunoassays and NAH assays).<sup>3,7</sup> The goal for the future is to improve the diagnosis of sexually transmitted infections by using multiplex tests, in particular DNA microarray technology.

### *Mycoplasmas*

These are the smallest free-living organisms capable of self-replication, and the genome of *M. genitalium* is the smallest of all Mycoplasma. The association of *M. genitalium* with human disease and genital tract disease in particular, was made possible after the development of PCR technology.<sup>8,9</sup> There is strong evidence that *M. genitalium* is associated with nonspecific urethritis in men, but there are not enough studies to support the contention that the bacterium can cause epididymitis and prostatitis and infertility.<sup>1,10,11</sup> PCR and serological studies of women have associated *M. genitalium* with PID, cervicitis, endometritis and infertility.<sup>9</sup>

**Laboratory diagnosis:** *M. genitalium* is difficult to study, because of their fastidious growth requirement, and its culture is difficult and, even when successful, it takes several weeks for each isolate to grow and identify. Serology in its more sophisticated forms may have a role in epidemiological studies but is not of value in clinical cases.<sup>12,13</sup> Nucleic acid amplification tests are the only available and reliable diagnostic tools for detection clinical disease due to *M. genitalium*, because of a very low load of mycoplasmas in some patients, tests with a very low limit of detection are needed in order to achieve sufficient assay sensitivity, but still there is no commercially available test has been released for diagnostic purposes.<sup>11,12</sup>

### *Ureaplasmas*

*U. urealyticum* is part of mycoplasma group. It is a commensal organism of the lower genitourinary tract of sexually active men

and women.<sup>14</sup> Ureaplasma infection in men is strongly associated with urethritis, prostatitis, epididymitis, whereas in women causes endometritis, chorioamnionitis, spontaneous abortion, prematurity/low birth weight, as well as arthritis and urinary calculi in susceptible adults.<sup>15</sup> Evidence has been presented that the species currently known as *U. urealyticum* should be separated into two new species, namely, *U. parvum* (previously *U. urealyticum* biovar 1) and *U. urealyticum* (previously *U. urealyticum* biovar 2). The exact role of *U. urealyticum* in male infertility remains a controversial subject as well as *M. genitalium*.<sup>16,17</sup>

**Laboratory diagnosis:** Ureaplasma infections have been traditionally diagnosed by culture, however, the culture is time consuming as it requires 2-5 days, whereas NAATs can detect their infections in few hours.<sup>17,18</sup> PCR-based methods are also becoming an important alternative to conventional culture for initial detection of ureaplasmas in clinical specimens and have the additional advantage of discrimination between the two Ureaplasma species.<sup>19</sup> Classic microbiological culture techniques are much less sensitive than PCR; less than half of the PCR positive probes in semen, urine, prostatic secretion, cervical swab, amniotic fluid, and vaginal specimen showed a positive culture.<sup>15,20</sup> The first PCR method for detection of human ureaplasmas in clinical samples was published in 1992.<sup>21</sup> Since then PCR methods have been increasingly used in the diagnosis of ureaplasma infections.<sup>1,21</sup> Sophisticated nucleic acid amplification tests are necessary to discriminate between the two *Ureaplasma* spp, this is the reason of the lack of species determination in most studies until the past few years.<sup>21</sup> Gene targets for PCR assays used to detect ureaplasmas and to define species and subtypes have included the subunits of urease gene, 16S rRNA genes and the multiple-banded antigen gene (MBA).<sup>19-21</sup> To characterize the ureaplasma species at the serovar level by PCR, genotyping primers based on the MBA gene and its 5'end upstream regions have been designed previously and partial serovar identification was achieved. In combination with direct sequencing or restriction enzyme analysis, these assays were capable of distinguishing ureaplasma serovars, and subtypes within serovars.<sup>19</sup>

Discrimination between harmless commensal colonization and clinically significant ureaplasma infection can be done by the application of quantitative PCR techniques and PCR serotyping. Real-time TaqMan PCR assays have been developed that allow rapid, specific, sensitive, and quantitative detection with a 100 times greater sensitivity than conventional PCR, using the same primer sets and cycling conditions.<sup>19,21</sup> Convenient differentiation of *U. parvum* and *U. urealyticum* is also possible with real-time TaqMan PCR or traditional PCR assays; it can also be used to discriminate among all of the serovars.<sup>1,21</sup> In conclusion, there is still a need to develop more easily and specific molecular methods for detection the majority of causative agents of sexually transmitted diseases.

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## Conflict of interest

Author declares that there is no conflict of interest.

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