CHAPTER I
INTRODUCTION

Chlamydia trachomatis and *Neisseria gonorrhoeae* infections belong to the most frequently encountered sexually transmitted infections worldwide (1). Even though the bacteriological diagnosis was not established until the 19th and 20th centuries, clinical symptoms probably associated with these bacteria are described in the ancient literature (2).

The clinical spectrum of genital diseases caused by these two bacteria is quite similar and mainly comprises urethritis in men and urethritis/cervicitis in women. However, no symptoms or only weak symptoms such as slightly discharge are common, especially in women. Oropharyngeal infections may occur in both sexes, and eye infections, acquired from infected mothers, are described in newborns. Conjunctivitis in adults is usually due to self-inoculation from a genital infection (3, 4).

Complications due to *C. trachomatis* and/or *N. gonorrhoeae* infections are mostly occurred in women. Ascending genital-tract infections involving the endometrium, fallopian tubes and/or adjacent pelvic structures, i.e. pelvic inflammatory disease (PID), may develop with a risk of infertility, ectopic pregnancy and chronic pelvic pain (5, 6). In addition to female morbidity, costs of these diseases are considerable (7). When the incidences of *C. trachomatis* and *N. gonorrhoeae* infections declined in worldwide, this was followed by declining incidences of PID and ectopic pregnancy (5, 6). Accordingly, strategies for detection, treatment and prevention of genital *C. trachomatis* and *N. gonorrhoeae* infections are essential to prevent PID and its later complications. In the absence of effective vaccines, the mainstay in the prevention of *C. trachomatis* and *N. gonorrhoeae* infections is the availability of specific laboratory diagnosis with high sensitivity, effective antibiotic treatment, contact tracing and screening of high incidence groups, as well as education of the public with respect to safe sexual behavior (5, 6, 8, 9).

Conventional diagnostic assays based on detection of live *C. trachomatis* organisms including cultivation in cell culture has been considered with high specificity and sensitivity under optimized condition. In this assay, specimen is collected in an appropriate transport medium before inoculated onto a confluent monolayer of McCoy cells (10). After 48–72 hours incubation, intra-cytoplasmic inclusions have been developed and detected by staining with fluorescein-conjugated monoclonal antibodies (mAbs) that are specific for the major outer membrane protein (MOMP) of *C. trachomatis* or other chlamydial specific proteins. Cell culture method is time consuming and laborious and was replaced by antigen- or deoxyribonucleic acid (DNA) / ribonucleic acid (RNA) detections in many routine laboratories during the 1990s. Some laboratories use antigen detection methods such as enzyme immunoassays (EIA) or direct immunofluorescent assays (DFA). EIA is targeted at the chlamydial lipopolysaccharide (LPS) by using specific monoclonal or polyclonal antibody. Some manufacturers such as MicroTrac and Chlamydiazyme have developed blocking antibody assay to verify *C. trachomatis*-positive EIA due to the potential for false-positive results. For DFA, specimen material is collected by placing directly on a slide. Fluorescein-conjugated antibody reacted with the Chlamydia surface antigens are visualized by fluorescence microscopy. Depending on the commercial products used, the antigen detected by the fluorescein-conjugated antibodies in the *C. trachomatis* DFA is either the LPS or the MOMP component. This method requires a skilled microscopist who can distinguish between fluorescing chlamydial particles and non-specific fluorescence. DFA is mostly used as a confirmatory test in some routine laboratories. In EIA tests, the anti-LPS antibodies can cross-react with *C. psittaci* and *C. pneumoniae* as well as nonchlamydial bacterial species. DFA with a *C. trachomatis*-specific anti-MOMP monoclonal antibody is considered as the highly specific assay (11).

Several commercial nucleic acid amplification tests (NAATs) are available at present. Like many in-house polymerase chain reaction (PCR) assays, they targeted at different regions of *C. trachomatis* DNA or RNA molecules in clinical samples. The major target sequences are located at the cryptic plasmid, which is present in high copy number, approximately 10 copies in each *C. trachomatis* organism (12, 13). Using this plasmid DNA as a target, the assay could theoretically increase the

sensitivity compared to assay using a single copy number of chromosomal gene such as the *omp1* gene (14). Commercially available NAATs for detection of the cryptic plasmid in *C. trachomatis* from clinical specimens are the COBAS AmplicorA *Chlamydia trachomatis* Test (Roche Diagnostic Systems), which is a PCR based-assay, the BDProbeTec^{TM}ET (Becton Dickinson), using strand displacement amplification (SDA) and LCx *C.trachomatis* A assay (Abbott Laboratories) using ligase chain reaction (LCR). However, the LCx *C.trachomatis* A assay is not available now. Comparison and evaluation of the performance of those NAATs assays have been done by Van Dyck et al. (15), and shows that the sensitivity and specificity were quite similar. The other NAATs such as Gen-Probe APTIMATM assay using transcription-mediated amplification (TMA) to detect a specific 23S ribosomal RNA (rRNA). This kind of target was present in hundreds of copies in each chlamydial organism. When comparison to the Abbott LCx and COBAS Amplicor assays in detection of *C. trachomatis* in the first void urine, the result obtained from Gen-Probe TMA assay was not significant difference from those two assays (16). In contrast, when comparing the NAATs assay with the non-amplification based assay such as PACE 2 DNA probe test from Gen-Probe, the sensitivity of amplification based-assay was significantly higher than the non amplification based assay in both endocervical swabs and urine samples (15, 18–24).

For *N.* gonorrhoeae, direct microscopic examination of Gram staining from genitourinary discharge is still being used in sexually transmitted diseases (STD) clinics. The test is rapid, inexpensive and highly specific. However, with the low sensitivity of the assay, persons with asymptomatic infection may be less likely to be detected (25–27). Cultivation is a gold standard method for diagnosis of gonorrhoea. Since the organisms are easily labile and fastidious in growing, the use of adequate swabs, a suitable transportation media and duration of transportation are of the utmost important keys of success. In addition, optimal inoculation and incubation as well as the use of selective culture media (28) combined with high quality nonselective media are also crucial (26, 29, 30). Under optimal conditions, cultivation of *N. gonorrhoeae* has high specificity and sensitivity, is inexpensive, and suitable for most types of specimens. Meanwhile, for diagnostic purposes it is quite time consuming. Different EIAs for antigen detection have also been developed for diagnosis of *N. gonorrhoeae*

infection. These are rapid, easy to perform, do not require viable organisms, and may be sensitive and specific for detection of bacteria in male urethral specimens and first-void urine (32). However, they are less sensitive for endocervical specimens (29, 33, 34) and in asymptomatic infection, and it is not possible to perform antibiotic susceptibility testing.

Numerous DNA/RNA-based noncultural methods, mainly NAATs, for detection of *N. gonorrhoeae* have been developed. The PACE 2 assay (Gen-Probe) with DNA probe hybridization has been used for several years to detect 16S rRNA of *N. gonorrhoeae* (35). Digene Hybrid Capture 2 (HC2) CT/GC including HC2 GC-ID for *N. gonorrhoeae* (Digene Corporation) is a newer RNA-probe based assay that identifies specific DNA sequences of the chromosome as well as the cryptic plasmid of *N. gonorrhoeae* (36). In general, the hybridization methods have a high specificity but a somewhat lower sensitivity than the NAATs. Over the years, different PCR based-assays have also been developed. Those assays targeted on *N. gonorrhoeae*-specific chromosomal genes encoding outer membrane proteins such as the reduction-modifiable protein (Rmp) (37), chromosomal putative cytosine DNA methyltransferase gene (Cobas Amplicor, Roche Diagnostics) (38, 39), *16S rRNA* gene (38), or the *cppB* gene located in regions of the cryptic plasmid that may also integrated into the chromosome (40). The LCRs (LCx, Abbott Laboratories) assay for detection of *N. gonorrhoeae* chromosomal genes, multicopy pilin and opacity-associated (Opa), have also been commercially developed (41, 42).

Although the nucleic acid-based methods have several advantages in diagnostic purpose especially the highly sensitivity and specificity as well as its rapidity and high throughput but almost all of them are available commercially with an expensive cost. This is a major limitation of the assays in diagnostic application. However, in an effort to prevent the spread of these diseases, increased attention is being focused on early diagnosis and treatment of both symptomatic and asymptomatic infected individuals.

Since *C. trachomatis* and *N. gonorrhoeae* are the most common STD in heterosexual males and females which cause about 40 to 50% of nongonococcal urethritis cases (44). Symptoms and complications from both organisms are similar and a substantial proportion of infected individuals (30-70%), especially women are

asymptomatic and serve as a reservoir of infection in communities (46). Treatment of infection is relatively simple and straightforward, with a 7-day course of doxycycline twice a day or a single dose of azithromycin for *C. trachomatis*, and a single dose of a quinolone or cephalosporin for *N. gonorrhoeae* (47-49). However, co-infection between these two organisms is also common, symptoms may overlap and making clinical diagnosis more difficult.

Standard laboratory diagnosis of these pathogens is based mainly on direct microscopy or isolation and identification of the viable organisms. Although they are relatively inexpensive and highly specific but logistically complicated and limited in sensitivity. A rapid, easily performed, and accurate diagnostic test are an urgent need to identify individuals who harbor these organisms. Accurately identification of individuals at risk for chlamydial or gonococcal infection is highly desirable facilitating early recognition and treatment of these infections and thus ultimately reduce reservoir of infectious pathogens.

Although the nucleic acid based test kits are commercially widely available, the routine use in diagnostic laboratory has been limited due to the budgetary constraints. In order to get an accurate diagnosis of *C. trachomatis* and *N. gonorrhoeae* infection, we evaluated the rapid and inexpensive multiplex single-tube nested PCR (M-SN PCR) for simultaneously detection of *C. trachomatis* and *N. gonorrhoeae* from different types of genitourinary specimen in a single reaction.

Objectives

1. To evaluate sensitivity of In-house multiplex single-tube nested polymerase chain reaction (M-SN PCR) in detection of *C. trachomatis* and *N. gonorrhoeae* DNA diluted in water or in clinical sample as a background of cellular DNA.

2. To evaluate the performance of In-house M-SN PCR in detection of *C. trachomatis* and *N. gonorrhoeae* in urine samples by comparing with Roche multiplex AMPLICOR CT/NG PCR.

3. To evaluate the types of genitourinary specimen suitable for detection of *C. trachomatis* and *N. gonorrhoeae* from both men and women by comparing between

urethral swab and urine from men and fresh cervical swab in transport medium and self-collected dry vaginal swab from women.

4. To analyze cost effectiveness of In-house M-SN PCR in detection of *C. trachomatis* and *N. gonorrhoeae* comparing with different commercial test kits.