CHAPTER V
DISCUSSION AND CONCLUSION

Over the past 15 years the diagnosis of *C. trachomatis* and *N. gonorrhoeae* infections has been largely dependent on traditional methods such as culture, enzyme immunoassay (EIA), and direct fluorescent antibody staining for *C. trachomatis* and Gram's staining, culture and biochemical tests for *N. gonorrhoeae*. For last 5 years, we have seen major improvements in ability to detect these STDs, first with the advent of newer technologies such as DNA hybridization and nucleic acid amplification and second with the testing of noninvasive urine specimens and self-collected dry vaginal swabs (14, 51, 108-111, 113-124). PCR and, more recently, ligase chain reaction (41, 113) have been developed for the detection of *C.trachomatis* and *N. gonorrhoeae*, and evaluations of the commercial test kits are now appearing (51, 108, 116, 117, 120). Nucleic acid amplification tests have generally been more sensitive than the traditional one (51, 114, 116, 117, 119-122). When applied to clinical samples, several recent studies demonstrated that PCR has higher sensitivity than culture for the detection of these two organisms. The sensitivity of culture has been estimated to range from 50-85 % in different laboratory setting compared to PCR assay. The over all sensitivity of PCR is ranging from 95-100 % when standard specimens; urethral swab and endocervical swab were used. However, with the alternative non-invasive samples; urine or vaginal swab, sensitivity was slightly lower 70-90%. The performance of the PCR technique used for diagnosis of C. *trachomatis* and *N. gonorrhoeae* infections may vary among different setting, format of assays and types of clinical specimen.

Different PCR systems including commercial and in-house PCR assays have been evaluated by many groups of study. Most of them have been shown to be highly sensitive for the detection of both C. *trachomatis* and *N. gonorrhoeae* in most clinical specimens (130). However, hindering factors like cost and specialized infrastructure have precluded the use of PCR for diagnostic purposes especially in developing countries. Although most commercial test kits for diagnosing C. *trachomatis* and *N.*

gonorrhoeae have been developed based on the convenient assay formats and have been available in many countries. However, the major drawback of their applications has been the budgeted spending for the assays.

We reported here the development of an inexpensive in-house M-SN PCR for the simultaneous detection of *C. trachomatis* and *N. gonorrhoeae* in genitourinary specimens. In general, SN PCR has the sensitivity as high as the conventional two tubes nested PCR but eliminates risk for contamination from the conventional assay. Moreover, with multiplexing format, our assay can detect both *C. trachomatis* and *N. gonorrhoeae* simultaneously from one sample and within one reaction. This makes the assay more convenience, less time consuming and save costs.

Before application further, the performance of our in-house assay had been verified against commercial test kit; Roche AMPLICOR CT/NG for detection of *C. trachomatis* and *N. gonorrhoeae* from urine samples. Different parameters; sensitivity, specificity, positive and negative predictive values were analyzed (table 3-4). As compared to the commercial test kit, our M-SN PCR had sensitivity for *C. trachomatis* and *N. gonorrhoeae* at 97% and 94% respectively. Samples that had been missed as false negative by M-SN PCR (2 samples for *C. trachomatis* and 1 sample for *N. gonorrhoeae*) were due to the low target DNA in the samples as determined by low O.D. measurement in amplicon detection step of Roche AMPLICOR CT/NG. However, specificity and positive predictive value for both organisms were 100% whereas the negative predictive value was 99%. According to the results above, our in-house M-SN PCR could be efficiently applied for clinical usage.

The assay developed in this study has lower detection limit at 10 pg and 1ng for *C. trachomatis* and *N. gonorrhoeae* DNA respectively when diluted with negative cervical swab extract. However, when diluted in water the detection limit was down to 1 pg and 100pg in a reaction for *C. trachomatis* and *N. gonorrhoeae* respectively. Although the detection limit obtained from our assay was higher than those reported by Mattony JB, et al. that their homebrewed multiplex PCR has detection limit at 10fg for both organisms (131). However, when applied for detection of the organisms from standard clinical samples; urethral and endocervical swabs, sensitivity of our assay was 83.8% and 100% respectively for *C. trachomatis* (table 5-6) and 93% and 100% respectively for *N. gonorrhoeae* (table 7-8). Specificity for both organisms was 100%

for both kinds of samples. This was equivalent to results reported by Mattony JB, et al. (131) that the sensitivity of the homebrewed multiplex PCR for *C. trachomatis* and *N. gonorrhoeae* were 100% and 93% respectively. The specificity was also 100% for both *C. trachomatis* and *N. gonorrhoeae*.

According to the preferential site of infections, urethral and endocervical swabs have been the specimens of choice for detection of *C. trachomatis* and *N. gonorrhoeae* in men and women. However, collection of these specimens is rather invasive and always denied by most patients. The alternative non-invasive specimens could eliminate this barrier. Many studies have aimed at urine and self-collected vaginal samples which are non-invasive in collecting. Moreover, specimen transportation is also one of the barriers for detecting the diseases, especially in remote area which had no medical facility. The dry form of specimens should be applied for this setting.

In order to eliminate those barriers, we evaluated the efficiency of corresponding non-invasive samples; urine and self-collected dry vaginal swab, for detection of *C. trachomatis* and *N. gonorrhoeae* by comparing with the standard samples; urethral and endocervical swabs. Although, non-invasive type of samples are mostly preferred by the patients and could substantially increase the acceptability and convenience for screening in a variety of settings. But some of them had limited in sensitivity of detection. For instance, self-collected dry vaginal swabs had lower sensitivity in detection of both *C. trachomatis* and *N. gonorrhoeae* than the standard endocervical swabs; 89-92% respectively (table 5-6). This may due to the limiting of infected cells obtained by this swab. Moreover, practicing variation from patient to patient can not be avoided. This seem to be the drawback for application of this samples, even though, they are more convenient in collection and transportation. To improve this, we need to well instruct women for self-collecting swab. However, others studies have reported that self-collected vaginal swab can be used successfully to diagnose sexually transmitted infections, eliminating the need for a clinician and a pelvic examination for specimen collection (132-135). These swabs have performed as well as or better than clinician-obtained endocervical swabs for diagnosis of either *N. gonorrhoeae* or *C. trachomatis* by DNA amplification assays. In 2002, Gaydos et al. (136) reported sensitivity and specificity for detection of *N. gonorrhoeae* in dry

vaginal swabs versus wet vaginal swabs was 88.9 % vs. 96.3% and 98.3% versus 98.2% respectively. And sensitivity and specificity for detection of *C. trachomatis* in dry vaginal swabs versus wet vaginal swabs was 91.3 % vs. 94.6% and 99.3% versus 99.3% respectively.

Self-collected dry vaginal swabs should be considered when it is desirable to test women who are not seeking health care but may have asymptomatic infections. For symptomatic women who can not obtain a clinical appointment immediately, self-collected dry vaginal swabs should also be considered as well. This could be beneficial to women in remote area. The availability of a self-administered specimen collection system would give women the opportunity to play a greater role in meeting their own health needs. Additionally, possibility to send self-collected dry vaginal swabs by post could open up an opportunity for new implication for large scale screening. Use these swabs might broaden access to new population groups, making it feasible to detect the infection in setting where the pelvic examination and cervical sampling are not routinely performed. It might also reduce the costs for screening programs for genital *C. trachomatis* and *N. gonorrhoeae* infections.

In men, urine samples are quite promising since they had higher sensitivity than the standard urethral swab for detection of both organisms; 89% vs. 84% for *C. trachomatis* and 98% vs. 93% for *N. gonorrhoeae*. Our finding had also confirmed the other studies reported elsewhere (51, 109, 116, 119, 120-122, 140-142). This may reflect the difficulty in obtaining sufficient *C. trachomatis* and *N. gonorrhoeae* or the infected cells from the urethral swab, since swab are inserted only 1-4 cm proximal to the meatus. In contrast to obtaining the urine sample, most of the microbes or infected cells were flooded thoroughly from proximal to distal tract. However, other studies are argued against these findings. Crotchfelt et al showed that, the sensitivity of urine was 91.1% compared with 99.3% for urethral swab (143). The study done by Wiesenfeld and colleagues gave a sensitivity of 98.4% for urethral swab and 87.1% for first void urine (144).

The need to develop acceptable, better, and more easily available techniques for diagnosing STD for all high-risk populations is an urgent issue in order to eliminate the reservoir and stop spreading of diseases. Additionally, STD is associated with increased risk for human immunodeficiency virus acquisition (138, 139). M-SN

PCR testing of FVU specimens and self-collected dry vaginal swabs should provide an improved method for diagnosis of both *C. trachomatis* and *N. gonorrhoeae* infections occurring either singly or as co-infections.

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 developing countries. Our in-house M-SN PCR is cost saver. The assay cost per test was about 189 Baht. It is 8-10 times less than the commercial tests. Moreover, the test is easy, the entire process of assay starting from DNA extraction amplification and detection can be carried by only one trained technician. In our hands, M-SN PCR can be completed in 4 hours and has a turnaround time of 1 working day. In-house M-SN PCR assay should provide powerful tools for diagnosing STDs including *C. trachomatis* and *N. gonorrhoeae* in clinical laboratories.

Our data suggest that self-collected vaginal swab transported in dry state has been efficiently used. Future studies to further explore the acceptability and usefulness of self-collected dry vaginal swabs should be undertaken to define their role in STD control in the large and variety group of women.