# AN ASSESSMENT AND EVALUATION OF METHODS FOR DIAGNOSIS OF CHLAMYDIAL AND GONOCOCCAL INFECTIONS

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**Abstract.** *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were studied in 350 females and 140 males attending the sexually transmitted disease clinic and AIDS Center, Khon Kaen zone 6 and the Division of Obstetrics and Gynecology, Khon Kaen Hospital. *Chlamydia trachomatis* infection was diagnosed by cell culture (CC), enzyme immunoassay (EIA) (Bioquest, NSW, Australia) and nucleic acid hybridization (PACE2 system : Gen-Probe, San Diego, Calif). It was found that the sensitivity, specificity and positive and negative predictive values in females were 95.7, 100.0, 100.0, 99.7% by the cell cuture; 91.3, 99.1, 87.5, 99.4% by the EIA; and 78.3, 99.7, 94.7, 98.5% by the PACE2 respectively. Values of the same parameters in males were 83.3, 100.0, 100.0, 98.5% by the cell culture; 75.0, 99.2, 90.0, 97.7% by the EIA and 91.7, 100.0, 100.0, 99.2% by PACE2 respectively. The methods for detection of *Neisseria gonorrhoeae* infection were conventional culture, PACE2 test and the direct examination (Gram's stain). In females, the sensitivity, specificity and positive and negative predictive values of the conventional culture were 85.7, 100.0, 100.0, 99.7% and those of the PACE2 were 85.7, 99.1, 66.7, 99.7% respectively. In males, the values of the same parameters were 81.8, 100.0, 100.0, 100.0% by the conventional culture, 95.5, 100.0, 100.0 and 99.2% by the PACE2. The prevalence of chlamydial infection in females was 6.6% (23/350) and that in males was 8.6% (12/140). The prevalence of gonococcal infection in females was 2.0% (7/350) and in males was 15.7% (22/140). The co-infection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in females was 0.9% (3/350) and no co-infection was found in males. It is concluded that cell culture is an appropriate method for detection of *Chlamydia trachomatis* in both genders, particularly in females. PACE 2 test is the best method for such detection in symptomatic males while EIA is a good method in females, particulary in symptomatic females. For gonococcal detection, PACE2 test is a sensitive, specific and alternative method to the conventional culture. It can be appropriately applied for the diagnosis of gonococcal infection, particularly in males.

## INTRODUCTION

*Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the most common causes of sexually transmitted diseases (STDs) worldwide and constitute a major public health problem in developing and industrialized countries (Sanders *et al*, 1994; Peeling, 1995). Both infections lead to costly acute illness, such as mucopurulent cervicitis in women and urethritis, prostatitis and epididymitis in men (Peeling, 1995). Asymptomatic women and men can contribute to transmission and to progression of these diseases (Sander *et al*, 1994; Quinn *et al*, 1996). Some cases result in serious complications such as pelvic inflammatory diseases (PID), ectopic pregnancy and tubal infertility (Ehret *et al*, 1993; Kluytmans *et al*, 1993; Peeling, 1995). Chlamydial infection during pregnancy may cause abortion or fetal abnormality and increase the risk for developing neonatal conjunctivitis and pneumonia (Schachter, 1989; Clark *et al*, 1992; Pearlman and McNeeley, 1992). For these reasons, screening for chlamydial and gonococcal infections to ensure early diagnosis and treatment is necessary.

In the case of chlamydial infection, cell culture has been used as the gold standard method for detecting infection in many studies. Its sensitivity has been reported from 33% to 95% when a single endocervical swab was tested (Ridgway, 1991; Thejls *et al*, 1994). Some studies indicated that the specificity and positive predictive value (PPV) of new tests were underestimated if culture was used as a reference (Thejls *et al*, 1994). This could result from a low sensitivity of the culture method. Disadvantages of the cell culture method are that it is labor intensive, time consuming and requires cold transportation (Barnes, 1989; Kluytmans *et al*, 1994). There are several assays for detection of chlamydial infection that are rapid and suitable for

automated high-volume testing such as enzyme immunoassay (EIA) and a nucleic acid hybridization assay (Barnes, 1989; Chapin-Robertson, 1993; Clarke *et al*, 1993; Ehret *et al*, 1993). Thus, one approach that can be applied to expand the reference method (instead of only the culture method) is to include two non-culture methods to discriminate between true positive and true negative tests, (Thejls *et al*, 1994). Futhermore, both of the non-culture assays should be compared with the cell culture and with each other because the non-culture methods detect different chlamydial target antigens.

Gonorrheal disease remains one of the most frequently occuring sexually transmitted diseases worldwide (Peeling, 1995) with a high number of cases reported in Thailand from the past until today (Venereal Disease Office, 1990-1996). The control of epidemic infection and treatment have depended on the diagnosis of *Neisseria gonorrhoeae* by culture technic. It is time consuming and depends on many factors for the success of culture (Hale *et al*, 1993; Vlaspolder *et al*, 1993). At this time, non-culture technics for the diagnosis of gonococcal infection are widely used (Vlaspolder *et al*, 1993) such as enzyme immunoassay (Stapinski *et al*, 1989) and nucleic acid hybridization (Hale *et al*, 1993; Panke *et al*, 1991; Vlaspolder *et al*, 1993). In this study, the nucleic acid hybridization (PACE2 assay) is an alternative technic applied for diagnosis of gonococcal infection in comparison with the conventional culture.

No basic epidemiological studies of chlamydial infection in males in Thailand has been published and no comparison between the diagnostic methods for chlamydial and gonococcal infection in Thailand has been evaluated. Therefore, the purpose of this study was to evaluate the standard cell culture (CC), enzyme immunoassay (EIA) and PACE2 systems for chlamydial infection and also to evaluate the conventional culture compared with the PACE2 in gonococcal infection in both genders. Futhermore, the prevalence of *Chlamydia trachomatis, Neisseria gonorrhoeae* and the co-infection of both microorganisms was included. The cost effectiveness of these methods applied for this study was also assessed.

## MATERIALS AND METHODS

**Patient population :** Endocervical swabs from 350 females and urethral swabs from 140 males were collected from the patients visiting Khon Kaen STD Clinic and AIDS Center, zone 6 and the outpatient clinic of the Division of Obstetrics and Gynecology, Khon Kaen Hospital. All of the samples were tested for both *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection. The patients who participated were interviewed with standard questionnaires which sought data regarding their age, urogenital symptoms, history of antibiotic administration and partner's health.

**Specimen collection and handling :** For female patients, one endocervical swab was taken and cultured on Thayer-Martin agar with antibiotics for culturing of *Neisseria gonorrhoeae.* Another 3 endocervical swabs were collected for the detection of *Chlamydia trachomatis*. One swab was placed into sucrose phosphate transport medium for chlamydial culture and another two swabs were for PACE2 and EIA. The two swabs were placed into the lysis buffer provided in the collection kits (the samples for PACE2 assay could be tested for both *Neisseria gonorrhoeae* and *Chlamydia trachomatis*). All samples kept in an icebox were sent to the laboratory and stored at -70°C until processing.

Male urethral samples for *Neisseria gonorrhoeae* culture were collected by using a sterile loop. For chlamydial detection, three urethral swabs were taken and manipulated as described for female samples.

**Direct examination :** Cervical swabs and discharge from urethral or urethral samples were tested with Gram's stain.

**Isolation and identification of *Neisseria gonorrhoeae* :** Culture was performed on a modified Thayer-Martin agar, incubated at 37°C for 48 hours with 5% $CO_2$. The isolate was identified as *Neisseria gonorrhoeae* on the basis of colony morphology, oxidase reaction and Gram's stain reaction (Gram negative diplococci with a coffee bean morphology). Some isolates were confirmed by carbohydrate utilization test.

**Cell culture (CC) for *Chlamydia trachomatis* :** *Chlamydia trachomatis* was cultured on cyclohexiimide-treated, McCoy cell as described previously (Ripa and Mardh, 1977). Briefly, 1 ml of vortexed specimen was inoculated on confluent McCoy cell monolayers on 12 mm round cover glass in a shell

vial and centrifuged for 1 hour at 35°C at 3,000 rpm. After centrifugation, 1 ml of maintenance medium (with 2 μg/ml cycloheximide) was added to each vial and further incubated at 35°C with 5% $CO_2$. After 48 to 72 hours, the monolayers were fixed for 10 minutes with 95% ethanol, stained with a fluorescein conjugated antichlamydial monoclonal antibody and examined for inclusion bodies under a fluorescence microscope. The CC with one or more fluorescent inclusion bodies was considered positive.

**PACE2 assay for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*** : The chemiluminescent probe assay utilized an acridimium ester labeled single stranded DNA probe(s) complementary to rRNA of *Chlamydia trachomatis* or *Neisseria gonorrhoeae*. It was performed according to the manufacturer's instruction. One positive and three negative references were included in each runing. Briefly, 100 μl of probe reagent (chlamydial or gonococcal DNA probe) was added to a tube containing 100 μl of vortexed specimen or control and incubated for 1 hours in a 60°C waterbath. One milliliter of separation solution (Containing metallic microbeads which bind hybridized probe) was added and mixed and the tubes were incubated in a waterbath (60°C) for 10 minutes. The rack of tubes was then placed on the base of the magnetic separation unit for 5 minutes at room temperature, and the supernatants were decanted. Washing solution was added, and after 20 minutes at room temperature on the magnetic separation base, the supernatants were decanted and light emission from the DNA-RNA hybrid was measured with a luminometer (Leader 50, Gen Probe, Inc, San Diego CA, USA). A sample was considered positive test if the difference between the Relative Light Units (RLUs) of the sample and the mean RLUs of negative reference was greater than or equal to 350 RLUs.

**EIA for *Chlamydia trachomatis*** : The HRP Chlamydia EIA (Bioquest, NSW, Australia) using a monoclonal antibody directed against a genus specific lipopolysaccharide (LPS) was applied. The procedure was performed according to the manufacture's instruction. Briefly, one hundred microliters of a sample or control were added to the antibody coated well and incubated at 37°C for 30 minutes. After 2 washing cycles, 25 μl of both horseradish peroxidase (HRP)-labeled monoclonal antibody and diluent buffer were added to each well. Six washing cycles were carried out, followed by incubating with 100 μl of 3, 3', 5, 5' tetramethyl benzidine (TMB) substrate at room temperature for 10-15 minutes. The substrate reaction was stopped with 50 μl of 1 M sulfuric acid. The color intensity was measured at 450 nm in a photometer. The sample absorbance was compared to that of the cut-off value. Samples with an absorbance value greater than the cut-off value were considered positive.

**Analysis of test results** : A sample was considered as a True Positive Test for either *Chlamydia trachomatis* or *Neisseria gonorrhoeae* when it showed (1) a positive standard cell culture (primary inoculation for *Chlamydia trachomatis*) or a positive conventional culture (for *Neisseria gonorrhoeae*) was observed and (2) negative culture but positive tests by both non-culture methods were observed. The sensitivity, specificity, positive and negative predictive values of the tests were analyzed in comparison with true positive tests.

## RESULTS

### Comparison of CC, EIA, and PACE2 for diagnosis *of Chlamydia trachomatis*

*Chlamydia trachomatis* was detected by CC, EIA and PACE2 in 22 (6.3%), 24 ( 6.9%) and 19 (5.4%) of endocervical samples and 10 (7.1%), 10 (7.1%) and 11 (7.9%) of male urethral samples, respectively. When the True Positive Test criteria was applied to the test results, it was found that the positive rates of chlamydial infection in females and males were 6.6% and 8.6 respectively (Table 1). Results of the three methods for chlamydial detection in females and males are shown in Table 2. Among these, 24 (4.9%) samples were found positive by three methods, 8 (1.6%) samples were found positive by at least two methods and 3 (0.6%) samples were found positive by the only CC method (Table 2). The sensitivity, specificity and positive and negative predictive values of each method for symptomatic and asymptomatic female and male patients are shown in Table 3. These results indicate that CC, and EIA are more specific and sensitive methods in symptomatic than in asymptomatic females, whereas the CC is probably the best methods applied in both symptomatic and asymptomatic females. For PACE2, it is the least sensitive method

Table 1

Positive rates of *Chlamydia trachomatis* infection as detection by EIA, CC and PACE2.

| Subjects (No.) | No. of positive sample (%) | | | True Positive Test* |
|---|---|---|---|---|
| | CC | EIA | PACE2 | |
| Females (350) | 22 (6.3) | 24 (6.9) | 19 (5.4) | 23 (6.6) |
| Males (140) | 10 (7.1) | 10 (7.1) | 11 (7.9) | 12 (8.6) |
| Total (490) | 32 (6.5) | 34 (7.0) | 30 (6.1) | 35 (7.1) |

* positive results according to True Positive Test criteria (see text).

Table 2

Number and percentage of endocervical samples and male urethral samples possesing *Chlamydia trachomatis* positive test by the three methods.

| Test methods and results | | | No. (%) | | Total |
|---|---|---|---|---|---|
| CC | EIA | PACE 2 | Females | Males | No. (%) |
| + | + | + | 17 (4.9) | 7 (5.0) | 24 (4.9) |
| + | + | - | 3 (0.9) | - | 3 (0.6) |
| - | + | + | 1 (0.3) | 2 (1.4) | 3 (0.6) |
| - | + | - | 3 (0.9) | 1 (0.7) | 4 (0.9) |
| + | - | + | - | 2 (1.4) | 2 (0.4) |
| + | - | - | 2 (0.6) | 1 (0.7) | 3 (0.6) |
| - | - | + | 1 (0.3) | - | 1 (0.2) |
| - | - | - | 323 (92.3) | 127 (90.7) | 450 (91.8) |
| | Total | | 350 (100) | 140 (100) | 490 (100) |

compared with CC and EIA in both symptomatic and asymptomatic females. However, it showed the best in sensitivity (91.7%) and specificity (100%) for chlamydial detection in male (Table 3).

**Comparison of culture, PACE2 and direct examination for diagnosis of *Neisseria gonorrhoeae***

Endocervical and male urethral samples were tested for gonococcal infection by conventional culture, PACE2 and direct examination. Table 4 shows the positive rate of *Neisseria gonorrhoeae* infection in females and males. The true positive test were samples yielding positive results according to True Positive Test criteria. *Neisseria gonorrhoeae* was detected by conventional culture, PACE2 and direct examination in 6 (1.7%), 9 (2.6%) and 3 (0.9%) of endocervical samples and 18 (12.9%), 21 (15.0%) and 21 (15.0%) of male urethral samples, respectively. When the True Positive Test criteria were applied to test results, it was found that the positive rates of gonococcal infection in females and males were 2.0% and 15.7% respectively (Table 4). Results of three methods in females and males are shown in Table 5. Among these, 18 (3.7%) samples were found positive by three methods, 10 (2.0%) samples were found positive by at atleast two methods and 1 (0.2%) sample was found positive by only the culture method (Table 5).

The sensitivity, specificity, and positive and negative predictive values of the culture and PACE2 for endocervical and male urethral samples are shown in Table 6.

Table 3

Sensitivity, specificity and positive and negative predictive values of the CC, EIA and PACE2 test compared with True Positive Test criteria in symptomatic and asymptomatic females and males.

| Gender (No.) | Status (No.) | Test | % Sensitivity | % Specificity | % PPV[a] | % NPV[b] |
|---|---|---|---|---|---|---|
| Females (350) | Symptomatic (181) | CC | 100.0 | 100.0 | 100.0 | 100.0 |
| | | EIA | 100.0 | 100.0 | 100.0 | 100.0 |
| | | PACE2 | 85.7 | 99.4 | 85.7 | 99.4 |
| | Asymptomatic (169) | CC | 93.8 | 100.0 | 100.0 | 99.4 |
| | | EIA | 87.5 | 98.0 | 82.4 | 98.7 |
| | | PACE2 | 75.0 | 100.0 | 100.0 | 97.5 |
| | Combined both clinical status | CC | 95.7 | 100.0 | 100.0 | 99.7 |
| | | EIA | 91.3 | 99.1 | 87.5 | 99.4 |
| | | PACE2 | 78.3 | 99.7 | 94.7 | 98.5 |
| Males (140) | Symptomatic* (116) | CC | 83.3 | 100.0 | 100.0 | 98.1 |
| | | EIA | 75.0 | 99.0 | 90.0 | 97.2 |
| | | PACE2 | 91.7 | 100.0 | 100.0 | 99.1 |

PPV[a]: positive predictive value; NPV[b]: negative predictive value.
* Chlamydia positive samples were found only in symptomatic men.

Table 4

Positive rates of *Neisseria gonorrhoeae* infection as detection by culture, PACE2 and direct examination (Gram stain).

| Gender | No. of positive sample (%) | | | True positive Test* |
|---|---|---|---|---|
| | Culture | PACE2 | Direct examination | |
| Females | 6 (1.7) | 9 (2.6) | 3 (0.9) | 7 (2.0) |
| Males | 18 (12.9) | 21 (15.0) | 21 (15.0) | 22 (15.7) |
| Total | 24 (4.9) | 30 (6.1) | 24 (4.9) | 29 (5.9) |

* positive results according to True Positive Test criteria (see text).

**Age and gender distribution of positive *Chlamydia trachomatis* and *Neisseria gonorrhoeae***

Age and gender distribution of *Chlamydia trachomatis, Neisseria gonorrhoeae* and co-infection are shown in Table 7. In female patients, the prevalence of *Chlamydia trachomatis* was 6.6% (3.9% in symptomatic and 9.5% in asymptomatic females) while that of *Neisseria gonorroheae* was 2.0% (2.2% in symptomatic and 1.8% in asymptomatic females). The co-infection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was 0.9% (1.1% in symptomatic and 0.6% in symptomatic females). In male patients, the prevalence of *Chlamydia trachomatis* was 8.6% (10.3% in symptomatic and none in asymptomatic males), that of *Neisseria gonorrhoeae* was 15.7% (18.1% in symp-

Table 5

Number and percentage of cervical and urethral samples possesing *Neisseria gonorrhoeae* positive test by the three methods.

| Test methods and results | | | No. (%) | | Total |
|---|---|---|---|---|---|
| Culture | PACE2 | Direct exam | Females | Males | No. (%) |
| + | + | + | 2 (0.6) | 16 (11.4) | 18 (3.7) |
| + | + | - | 3 (0.9) | 1 (0.7) | 4 (0.8) |
| + | - | + | 0 (0) | 1 (0.7) | 1 (0.2) |
| + | - | - | 1 (0.29) | 0 (0) | 1 (0.2) |
| - | + | + | 1 (0.3) | 4 (2.9) | 5 (1.0) |
| - | + | - | 3 (0.9) | 0 (0) | 3 (0.6) |
| - | - | - | 340 (97.1) | 118 (84.3) | 458 (93.5) |
| | Total | | 350 (100) | 140 (100) | 490 (100) |

Table 6

Sensitivity, specificity and positive and negative predictive values of the culture and PACE2 test compared with the True Positive Test criteria for diagnosis of *Neisseria gonorrhoeae* infection.

| Gender | Methods | % (no. of positive samples/no. tested) | | | |
|---|---|---|---|---|---|
| | | Sensitivity | Specificity | PPV[a] | NPV[b] |
| Females | Culture | 85.7 (6/7) | 100.0 (343/343) | 100.0 (6/6) | 99.7 (343/344) |
| | PACE2 | 85.7 (6/7) | 99.1 (340/343) | 66.7 (6/9) | 99.7 (340/341) |
| Males | Culture | 81.8 (18/22) | 100.0 (118/118) | 100.0 (18/18) | 100.0 (118/118) |
| | PACE2 | 95.5 (21/22) | 100.0 (118/118) | 100.0 (21/21) | 99.2 (118/119) |

PPV[a]: positive predictive value; NPV[b]: negative predictive value.

tomatic and 4.2% in asymptomatic males. However, the co-infection was not found in males. The age group with the highest infection rate of both organisms in both genders was 15-24 years (Table 7).

## DISCUSSION

Three diagnostic methods, cell culture, EIA and PACE2, for chlamydial infection were performd. The cell culture seemed to be the best method in both symptomatic and asymptomatic female patients. The EIA was considered the best method for symptomatic females, like the cell culture but its sensitivity and positive predictive value (PPV) showed that the sensitivity of EIA for males samples was lower than that of other methods (Table 3). The greater sensitivity of EIA in women rather than in men was probably due to the fact that median number of inclusion forming units in cervical samples was typically 10-fold higher than that found in urethral samples (Clarke *et al*, 1992). This result supports the study by many investigators in which the EIAs were not as sensitive and specific as culture technic in males and in asymptomatic or low prevalence female population (Chernesky *et al*, 1986; Stamm, 1988). The lack of specificity would result from cross reaction with other organisms, such as Gram negative bacteria (Stamm, 1988; Ridgway and Taylor-Robinson, 1991). However, in this study we found that the specificity of EIA was quite good even though it yielded more false

Table 7

Age and gender distribution of *Chlamydia trachomatis, Neisseria gonorrhoeae* and their co-infection in symptomatic and asymptomatic subjects as assessed by True Positive Test described in text.

| Age group | Females (No. positive/No. tested) | | | Males (No. positive/No. tested) | | |
|---|---|---|---|---|---|---|
| | CT[1] | NG[2] | Co-in[3] | CT[1] | NG[2] | Co-in[3] |
| 15-24 | 10.8 (17/158) | 3.2 (5/158) | 1.3 (2/158) | 10.6 (5/47) | 25.5 (12/47) | 0.0 |
| 25-34 | 2.9 (4/137) | 1.5 (2/137) | 0.7 (1/137) | 9.8 (5/51) | 13.7 (7/51) | 0.0 |
| 35-44 | 5.3 (2/38) | 0.0 | 0.0 | 6.7 (2/30) | 6.7 (2/30) | 0.0 |
| > 45 | 0.0 | 0.0 | 0.0 | 0.0 | 8.3 (1/12) | 0.0 |
| Total | 6.6[(a)] (23/350) | 2.0[(b)] (7/350) | 0.9[(c)] (3/350) | 8.6[(d)] (12/140) | 15.7[(e)] (22/140) | 0.0 (0/140) |

[(a)] 3.9% (7/181) symptomatic and 9.5% (16/169) asymptomatic subjects
[(b)] 2.2% (4/181) symptomatic and 1.8% (3/169) asymptomatic subjects
[(c)] 1.1% (2/181) symptomatic and 0.6% (1/169) asymptomatic subjects
[(d)] all (10.3%, 12/116) were symptomatic subjects
[(e)] 18.1% (21/116) symptomatic and 4.2% (1/24) asymptomatic subjects
CT[1] = *Chlamydia trachomatis* (positive tests included co-infection)
NG[2] = *Neisseria gonorrhoeae* (positive tests included co-infection)
Co-in[3] = co-infection of both *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

positive results than other methods [1 case in men (0.7%) and 3 cases in women (0.9%)]. The differences in this EIA result compared with the EIA results of other previous reported tests may be due to several factors, one of which is the measurement of different antigens by each EIA test. The EIA test of the Bioquest company employs a murine monoclonal antibody to lipopolysaccharide (LPS) and measures only LPS antigen which is similar to IDEIA. Other EIAs test (such as Chlamydiazyme) employ a polyclonal antibody and should be capable of measuring several antigenic components.

The PACE2 assay was the best method compared with cell culture and EIA for chlamydial detection in male patients (Table 3). However, it sensitivity was not satisfactory in asymptomatic females. The decrease of sensitivity might be caused by an insufficient amount of chlamydial rRNA to be detected (Kluytmans *et al*, 1991). Some reports found that when the number of inclusion bodies was less than 5-10, the sensitivity will be affected (Chapin-Robertson, 1993). All researchers, however, were concerned for RLU values when the samples were expressed as borderline values (Chapin-Robertson, 1993). Some reports suggested that if the sample RLU : cut off RLU ratio was between 0.7-3.0, the sample should be retested (Yang *et al*, 1995). Similary, in this study, the samples were confirmed when they were expressed as boderline values as recommended above.

The differences in the sensitivity in each method may depend on several parameters including type and site of specimen, gender, prevalence, number of samples collected, type of swabs used, transportation and storage conditions, laboratory skills and amount of antigen present in the clinical specimens (Mahony, 1989). The use of so many swabs may affect the number of organisms which would be present in one swab but not in another, especially in latent or asymptomatic infection which were resulting in a low level of infectious organisms (Clark *et al*, 1992; Ehret *et al*, 1993; Thejls, *et al*, 1994). This, together with the variation in swabs collected by many physicians, makes the sampling process highly randomized (Mahony *et al*, 1985; Iwen *et al*, 1991). However, some studies found that the swab

order was not a significant factor (Clarke *et al*, 1993; Warren *et al*, 1993). Other factors, such as specimen transportation and storage conditions, the prior use of antibiotics and prevalence of infection in the tested population also influenced the test results. The prevalence of *Chlamydia trachomatis* infection in males (8.6%) was higher than that in females (6.6%) and all of the chlamydial positive samples from males were taken from symptomatic patients who requested effective treatment of the disease. In contrast, infection in females was often asymptomatic, resulting in an inappropriate treatment, a major factor accounting for spreading of this disease. Sometimes infection could progress to serious complications of the urogenital organs. Thus, screening for chlamydial genital infection was important for the female population at risk.

The study for *Neisseria gonorrhoeae* infection revealed that PACE2 was less sensitive than previous studies in female patients (Hale *et al*, 1993; Vlaspolder *et al*, 1993). However, in male patients, the values of sensitivity, specificity and predictive values of PACE2 were high. There were 4 culture negative but PACE2 positive results from both male and female patients (Table 5). All of the four men stated clinical symptoms, direct examination showed Gram negative diplococci, and it was noticed that all of them used antibiotics which could affect culture results. Three of four female patients also gave clinical symptoms. One of them did not mention symptoms but her husband presented symptoms of discharge. The 3 symptomatic females used antibiotics before attending the STD clinic. Although 3 of the female samples were negative in both culture and direct examination, for PACE2 assay, those 3 samples showed sample RLU: cut off RLU ratios higher than 10. Thus, taking everything into consideration, it is possible that the 3 endocervical samples may be false negatives by culture and the direct examination. According to criteria in this study, these 3 samples were considered as the negative tests. The overall, PACE2 is more sensitive method for detection *Neisseria gonorrhoeae* than culture in males but it is similar in females. This agrees with Vlaspolder *et al* (1993) who suggested that the PACE2 DNA probe assay might be a more sensitive than culture for detecting gonococcal infection and showed that no false positive reactions of PACE2 was seen after adequate patient treatment.

In this study, co-infection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in male patients was not observed but it was found 0.9% (3/350) in female patients or 11.1% (3/27) in female infections. This corresponds with the result of Tapsall *et al* (1996) who found that co-infection in women was notable higher than in men. Another reason may be the number of male subjects was too small.

The advantages of PACE2 assay over the culture method for gonococcal detection is that the PACE2 swab requires no special handling, the organisms can survive for prolonged storage and samples can be frozen with no effects on cultivation later. The success of culturing depends on many factors such as accurate monitoring of incubation temperature, proper $CO_2$ concentration and immediate incubation of the plate after inoculation. However, an important disadvantage of PACE2 system is the high price per test.

The commercial sex workers and laborers had the highest prevalence of both *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in female and male patients, respectively (data not shown). However, previous results in Thai female sex workers reported by Nirothisard *et al* (1991) seemed to be different from those reported in this study in which the positive rate was lower. It might imply that the sexual behavior has changed because of AIDS prevention programs. In addition, the results from this study indicated that young, sexually active males and females (15-24 years) had the highest infection rate of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. It was also observed that the STDs *Neisseria gonorrhoeae* and *Chlamydia trachomatis* tended to spread to the housewife group (data not shown). Regarding the prevalence of chlamydial and gonococcal infections in symptomatic and asymptomatic patients, it was found that both bacterial infections were more symptomatic in males than in females. This result corresponded to Sanders *et al* (1994).

In conclusion, cell culture seemed to be an appropriate test for chlamydial detection in both males and females particularly, in symptomatic females. EIA was suitable for females, particularly in symptomatic females but not for male patients whereas PACE2 was the most appropriate method for chlamydial infection in males but not in females. However, the selection of a test method for chlamydial diagnosis should be done with the understanding that no one method is perfect. The

culture should be used in an asymptomatic or low prevalence population but it is technically difficult, requires good laboratory facilities, is time consuming and costly. The EIA and PACE2 are useful for high-volume screening and can provide results with a shorter turnaround time. Futhermore, another advantage of PACE2 assay is the ability to test a single specimen for both gonococcal and chlamydial infection in parallel tests.

Moreover, the cost effectiveness for each method should be considered. For a chlamydial test, EIA is the most cost effective assay compared with the other two methods. The price is approximately US$3 (however the cost is approximately US$3-6 when other companies kits are used). The price per test for CC is approximately US$5 and for PACE2 assay is US$6. For *Neisseria gonorrhoeae* test, the list price for a Thayer-Martin plate plus antibiotics is approximately US$1.5 compared with the list price for the PACE2 assay, which is approximately US$4. Therefore, the cost may be viewed as a disadvantage of the PACE2 assay for detection of both microorganisms.

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