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A Comparison of First-Void Urine, Self-Administered Low Vaginal Swab, Self-Inserted Tampon, and Endocervical Swab Using PCR Tests for the Detection of Infection with *Chlamydia Trachomatis* in Commercial Sex Workers and Women Attending Gynecology Outpatients in Southern Thailand

Verapol Chandeying,*
Steven Skov,**
David Farrell,***
Surachai Lamlertkittikul,****
Putchart Lamlertkittikul,****
Sepehr Tabrizi,*****
Jintana Pradutkanchana J,******
Roongrueng Jarumanokul,******
Oranart Suddeaugrai.******

**Department of Obstetrics and Gynecology, Faculty of Medicine,*
***Medical Officer, AIDS/STD program, Darwin, Australia,*
****Queensland Health Pathology Service, Toowoomba Laboratory, Toowoomba, Australia,*
*****Hat Yai Center Hospital, Hat Yai, Songkla, Thailand,*
****** The Royal Women's Hospital, Melbourne, Australia and,*
*******Department of Virology and Immunology, Prince of Songkla University, Hat Yai, Songkla, Thailand*

ABSTRACT

Objective To compare the ability of different specimen types, endocervical swab, first-void urine, self-inserted tampon and self-administered low vaginal swab, using polymerase chain reaction (PCR) tests to detect infection with *Chlamydia trachomatis* and to assess the acceptability of the specimen types to women.

Subjects Two cohorts of women in Songkla province southern Thailand: 501 commercial sex workers (CSWs) presenting to the VD units in Hat Yai, and 405 women attending gynecology outpatients at the Hat Yai provincial hospital.

Methods As part of a gynecological assessment, four specimens were collected to be tested by PCR for *Chlamydia trachomatis*: first void urine, self-administered low vaginal swab, self-administered tampon and an endocervical swab. Before and after the examination questionnaires were administered concerning the women's sexual health, previous use of tampons and their willingness to use similar specimens if necessary in the future.

Results The prevalence of infection with *C. trachomatis* amongst the CSWs was 17.6% and 5.7% amongst the outpatient women. Acceptance of the tampon is 72.6%, and self administered vaginal swab is 74.2%. In CSWs the tampon was the most sensitive method (95.9%) for detecting infection followed by the self-administered low vaginal swabs (89.2%). There was no statistically significant difference between these two specimens but both were significantly more sensitive than either urine or endocervical swabs. In outpatient women, the endocervical swabs had the most sensitivity (100%), while the tampon, self-administered low vaginal swab had very similar sensitivities, both were 85.7%. Specificities for all specimens were in excess of 98% with the tampon being slightly less specific than other specimens in both CSWs and outpatient women.

Conclusions Tampons and self-administered low vaginal swabs (LVS) have high sensitivity for detection of *C. trachomatis* and appear acceptable to women in southern Thailand. Sensitivity of first void urine is lower, but still high enough to be useful in certain circumstances. Self-administered specimens offer many possibilities for routine diagnosis, research and population interventions and could contribute to reductions in STDs, their sequelae and HIV transmission at a population level.

Key words: polymerase chain reaction, chlamydia, endocervical swabs, first-void urine, tampon, low vaginal swab, commercial sex workers, gynecology outpatients

Since the advent of the nucleic acid amplification (NAA) techniques as diagnostic tools for STD detection, many new possibilities have emerged for both research and STD control strategies. The sensitivities and specificities of these techniques are high and can be superior to those of standard techniques. Also, specimens tolerate a wider range of conditions and time delays during transport from the site of collection to laboratory.[1-10] Importantly, these technologies have proved particularly useful for simultaneous detection of a number of genital tract infections, especially *C. trachomatis* and *N. gonorrhoeae*, in first-void urine samples or self-administered tampons or low vaginal swabs in women.[11-18] The non- or minimally invasive nature of urine, tampon or low vaginal swab specimens offers enormous advantages over endocervical swabs both in terms of acceptability to clients and in reducing the need for the trained personnel and material required for pelvic examinations.

NAA tests could therefore be extremely useful in service provision, research or population interventions for genital infections in women. The question arises as to what is the best specimen to use for the detection of genital infections in women. This will vary according to the context and purpose of doing the test, local cultural sensibilities, the level of training of health personnel, the costs involved as well as the sensitivities and specificities of the various specimens. The acceptability to women of self-administered tampon or low vaginal swabs may vary in different cultures. Many workers, particularly in developing countries, may not be familiar with the use of these tests and these specimens and work remains to be done to further demonstrate their technical characteristics and usefulness in a practical situation.

The aim of this study was to compare several specimen types in the same women using using polymerase chain reaction (PCR) to detect infection with *Chlamydia trachomatis*. The study also aimed to assess the acceptability to the women of these specimen types.

Methods

The study received approval from the Prince of Songkla University Ethics Committee. Two groups of women were invited to participate: commercial sex workers (CSWs) presenting to the Hat Yai VD unit either for a routine check-up or with symptoms and women presenting to the Hat Yai provincial hospital gynecology outpatients department.

After the study was explained and verbal consent obtained, the women were asked to complete a questionnaire asking whether they had ever had a Pap smear, ever used tampons before or ever had

an STD. Clinical assessment, including history, examination, and investigations, including an endocervical swab for PCR testing, as appropriate to their particular situation was then performed. The examinations were performed by clinic nurses in the VD unit and by gynecologists in the outpatient department. Before the pelvic examination they were asked to pass a first void urine, do a self-administered low vaginal swab and also insert and withdraw a tampon. Clear instructions were given by the research staff in how to use the swab and tampon and the women were asked to leave them in place for about five seconds. The vaginal swab, the tampon, and the endocervical swab were placed in separate containers of 2 sucrose phosphate (2SP) solution for transport to the laboratory. Urine specimens were placed in a refrigerator at 4-8˚C. After the specimens were obtained, the women were asked whether they would be willing to use these specimen types in the future. Women received standard management and follow-up according their circumstances and the clinical protocols in use at the clinics.

Within six hours of collection, specimens for PCR testing were transported to the Prince of Songkla University microbiology laboratory and frozen at -70˚C until PCR testing could be performed. All specimens were tested for *C. trachomatis* using the Roche AMPLICOR system (Roche Diagnostics, Branchburg, NJ, USA) according to the manufacturer's instructions.

From all women who had a positive result and from a random selection of 10% of women who had a negative result, an aliquot of specimen fluid was sent on dry ice to Toowoomba, Australia. In the majority of instances where the tampon specimen was positive, this was the specimen used for this purpose. Where the tampon specimen was negative, an aliquot from the specimen which was positive was used. This aliquot was then re-tested using an alternative PCR assay targetting the MOMP gene.[19]

A positive result was considered a true positive if more than one specimen site was positive. In those women in whom only one specimen was positive, it was considered a true positive if the alternative PCR test in Toowoomba was also positive.

Statistical analysis for means and comparisons of means was done using EpiInfo version 6. McNemar's test was used to compare the differences in sensitivity and specificity of the various specimen types.

Results

Study participants

During the study 1,011 women were invited to participate of whom 953 in 1,011 (94.3%) agreed to do so: 523/566 CSWs, and 430/445 out patient women (p = 0.6). The majority of study participants, 894 (93.8%), were Buddhist with 59 (6.2%) being Muslim. There was no difference between the participants, in their mean age, 27.8/26.9 years (p=0.3), their histories of never used tampon, 90.2/86.2% (p=0.3), or of ever having had a pap smear, 44.2/39.7% (p=0.6), or an STD, 12.5/10.3% (p=0.8). Of the study participants, the CSWs were slightly younger, used tampons more, and more often had a history of an STD compared to the outpatient women (Table 1).

Table 1. Participants' age, history of pap smear, STD and tampon use by type of participant

	CSWs n = 523	GYN outpatients n = 430	p values
Mean age (range)	26.8 yrs (16-52)	28.9 yrs (15-47)	<0.001
Ever had pap smear	212 (40.5%)	209 (48.6%)	0.015
Ever had an STD	102 (19.5%)	17 (4%)	<0.001
Never used tampons	449 (85.8%)	411 (95.6%)	<0.001

Of the 58 in 1,011 women who declined to take part, 35 (60.3%) did so because they had never used a tampon, and 17 (29.3%) because of fear of pain or discomfort while the remaining six (10.3%) gave no reason.

The overwhelming majority of CSWs (518/523: 99%) who took part presented to the clinic for a routine check-up. In contrast, only 21 of the 430 outpatient women (4.9%) did so with the great majority presenting with symptoms. The reasons for their presentations to the gynecologic clinic were: pelvic discomfort (152/ 430, 35.3%), amenorrhoea (136/430, 31.6%), postpartum examination (84/430, 19.5%), leucorrhoea (13/430, 3%), and genital rash (4/430, 0.9%), respectively.

Three specimen types (tampon, urine and endocervix) were collected in 906 of the 953 women (95.1%): 501 CSWs (96%) and 405 outpatient women (96%). All four specimen types were collected in 441 CSWs and 129 outpatient women.

Willingness to re-use specimens

When asked whether they would be willing to use either a self-administered tampon or low vaginal swab in the future, approximately three-quarters of women were willing to do so. More outpatient women were willing to use a tampon specimen again than were CSWs. (Table 2)

Table 2. Willingness to re-use specimens in future

Specimen type	All women	CSWs	Gyne outpatients	p values: CSW vs outpatients
Tampon	72.6%	62.3%	85.1%	0.002
Self-admin LVS	74.2%	74%	75.2%	0.97

Prevalence of infection

Eighty-eight in 501 CSWs (17.6%) were found to be infected according to our "modified gold standard" as were 23 in 405 (5.7%) of outpatient women. As there was variation in the numbers of specimens women had collected, the data were analysed to describe the sensitivity and specificity results. The frequency of a positive result according to the Roche AMPLICOR for the various specimen types and the frequency of one or more specimen types being positive in the same woman are shown in table 3.

Table 3. Crude frequency of positive results by specimen type

Type of specimen	C. trachomatis positive	
	CSWs no. (%)	Outpatient women no. (%)
Roche AMPLICOR results		
Self-inserted tampon	88/501 (17.6%)	29/405 (7.2%)
Self administered low vaginal swab	69/441 (15.6%)	7/129 (5.4%)
First void urine	64/501 (12.8%)	17/405 (4.2%)
Endocervical swab	58/501 (11.6%)	23/405 (5.7%)

Fluid from two hundred and twenty-one specimens, were re-tested in Toowoomba using an alternative assay targeting the MOMP gene. From CSWs there were 134 tampon specimens (84 positives and 50 negatives), 6 endocervical specimens (4 positives and 2 negatives) and 8 urine specimens (4 positives and 4 negatives). From outpatient women 65 tampon specimens (28 positives and 37 negatives), 5 endocervical specimens (5 negatives) and 3 urine specimens (3 negatives) were re-tested. (Table 4)

Of the twenty-eight CSW specimens which were MOMP assay negative but considered a "true positive", there were seven with four specimens positive, eleven with three and ten with two positive. Similarly three of four positive endocervical specimens and two of four urine specimens positive initially were not confirmed by the MOMP assay. One each of these was considered a true positive by dint of another specimen from the woman being positive.

Table 4. Result of confirmatory testing with MOMP gene PCR

Specimen	Initially positive	MOMP negative	MOMP negative but confirmed by other specimen type	completely unconfirmed
CSW tampon	84*	34	28*	6
Outpatient tampon	28	7	2	5
CSW endocervix	4	3	1	2
CSW urine	4	2	1	1
Total	120	46	34	12

*Not all positive specimens were able to be sent for confirmatory testing

The sensitivities and specificities for the various specimen types in CSWs and outpatient women are displayed in tables 5 and 6. There was no significant difference in sensitivity between tampons and low vaginal swabs in CSWs but both of these were significantly more sensitive than either urine or endocervical swabs. Amongst outpatient women there were no statistically different sensitivities observed.

Table 5. Sensitivity and specificity of specimen types in CSWs according to modified "gold standard"

	Sensitivity	Specificity
Tampon	71/74 = 95.9%	361/367 = 98.4%
Urine	52/74 = 70.3%	366/367 = 99.7%
Endocervical swab	44/74 = 59.5%	366/367 = 99.7%
Vaginal swab	66/74 = 89.2%	364/367 = 99.2%

Table 6. Sensitivity and specificity of specimen types in outpatient women according to modified "gold standard"

	Sensitivity	Specificity
Tampon	6/7 = 85.7%	121/122 = 99.2%
Urine	5/7 = 71.4%	122/122 = 100%
Endocervical swab	7/7 = 100%	122/122 = 100%
Vaginal swab	6/7 = 85.7%	121/122 = 99.2%

Discussion

In this study, our definition of a "true positive" was if more than one specimen from a woman was positive by the Roche AMPLICOR. In general, the Roche AMPLICOR assay for chlamydia is considered to have very high specificity and standard laboratory practice for routine diagnosis. Care was taken to avoid contamination from one specimen type to another.

We believe that the amount of infection detected closely reflects the reality. The prevalences of chlamydial infection observed, 17.6% in CSWs and 7.2% in outpatient women, are in keeping with prevalences observed elsewhere in Thailand. A study of outpatient women with vaginal discharge in Hat Yai using Enzyme Immunoassay (EIA) found that 4% were infected.[20] Prevalences of 4.6% and 6.8%[21,22] using PCR and EIA tests have been reported in rural village women and 5.7% (PCR) and 9.1% (GenProbe) in antenatal women.[23,24] Amongst sex workers, prevalences of 17-20% have been described.[25,26]

The prospect of using tampons or self-administered vaginal swabs as specimens in populations who are not familiar with their use raises concerns about whether they are culturally appropriate. However, tampons have been found suitable and acceptable for collecting genital specimens in virginal women[27] and amongst Aboriginal women living a near-traditional lifestyle in remote communities in Northern Australia.[12,13] Several studies of self-administered vulval or low vaginal swabs have had very high participation rates and not reported any difficulty in acceptability to the women taking part.[14-17] One study reported that while 42% of women preferred to give a urine sample, 53% found a urine or self-administered vaginal swab to be equally acceptable.[18] In our study, 94% of women agreed to participate in the study and 75% said they would be willing to use either a tampon or self-administered vaginal swab again. There are many situations when a pelvic examination is not altogether necessary or practicable. For example, where there is not a health care provider available who is trained in pelvic examination, an asymptomatic woman who is seen as part of contract tracing but is unwilling to have a pelvic examination, during a cross-sectional prevalence study or a community mass screening program in asymptomatic women. In these circumstances it is likely women may prefer a test they can do themselves, as is the experience in Northern and Central Australia where self-administered tests are now widely accepted amongst both women and health staff.[28,29] This innovation has greatly improved the access that women have to diagnostic tests; even to the point that, at times, they are used when a pelvic examination and endocervical swabs are clinically indicated and to be preferred.

We observed some variation in the sensitivities and specificities of the specimen types were considered in the analysis. However, none of these differences were statistically significant. This may be a result of the "gold standard" used whereby positive results in two different specimens "confirmed" each other. With the extra specimen, there is a greater likelihood of this occurring.

Overall, we found that the tampon had the greatest sensitivity in detecting infection followed by the low vaginal swab. Both of these specimen types had very high sensitivity very similar to those observed

in other studies of self-administered vaginal specimens.[14-18] In CSWs both of these specimens had a signficantly greater sensitivity than either urine or endocervical specimens. In the outpatient women the performances of the tampon and endocervical swab were nearly identical but the difference between tampon and urine was nearly but not quite significant. The lower sensitivity of endocervical swabs in the CSWs may reflect a higher degree of training and experience in collecting endocervical swabs amongst the gynecologists as opposed to the nurses in the VD clinics. Other studies have found introital swabs to have virtually equivalent[14,16-18] or even greater sensitivity[15] to that of endocervical swabs for the detection of *C. trachomatis*. The urine specimens were the least sensitive as has been found elsewhere, but still detected a large number of infections.[13,15,18]

The sensitivity of the self-administered vaginal swab in our study was slightly lower than that of the tampon. This difference was not statistically significant, but the study did not have the power to detect small differences in sensitivity. One might expect a tampon to detect more infections because of the larger amount of fluid and cells it will absorb. Whatever difference which might exist is probably small. We found the specificity of tampons to be slightly lower than the other specimens. Tampons may be more susceptible to cross-contamination both in the field because their absorbent surface is handled directly by the women who use them and in the laboratory because of the more complicated procedure necessary to extract fluid from them.

However, sensitivity and specificity should not be the sole factors considered in choosing a specimen for a particular application. Certainly this study and others illustrate one of the particular advantages of NAA technology; that is, the reduced need for trained personnel and all that is necessary for an pelvic examination, in order to obtain an adequate genital swab.

If self-administered swabs with a "standard" swab were more acceptable to the client population or easier or cheaper to use in the field or the laboratory, they might then be preferable to tampons. And, although urine specimens seem less sensitive than the other specimens, they still detect a large number of infections and their ease of use and acceptability to women make for a very useful health program tool. For example, Nganampa Health Council in South Australia have achieved substantial reductions in the prevalence of both gonorrhoea and chlamydia in men and women in remote Aboriginal communities with a systematic screening strategy using PCR tests of urine samples.[28,29]

Although NAA tests are more expensive than EIA, the benefits may outweigh the costs even in some developing nations. The role of asymptomatic carriers of infection in propogating the spread of infection in a population is well known. Targeted screening of women to detect cervical chlamydial infection has been shown to decrease the incidence of symptomatic PID.[30] Gonococcal or chlamydial infections increase the risk of HIV transmission[31] and improving access to STD diagnosis and treatment has been shown to reduce the incidence of HIV.[32] NAA tests can enhance the acceptability of and access to testing and increase detection of infection amongst those tested. By their use, health services could make real gains in reducing the enormous human suffering and financial costs of long-term reproductive sequelae in women and HIV infection.

In summary, NAA tests provide highly sensitive and robust means to detect sexually transmitted infections both in the clinic and field conditions. Our study found that tampon specimens had the highest sensitivity of the four specimen types albeit with a lightly lower specificity. Both tampons and self-administered vaginal swabs were acceptable to the majority of women. Non- or minimally invasive specimens using NAA technology offer many new possibilities to improve health care seeking behaviour and for practically orientated research such as prevalence studies or to develop clinical algorithms. The control of STDs in a population would be enhanced by sensitive self-administered methods for the detection of asymptomatic infection. An

understanding of the relative advantages of different specimen types will allow program planners to determine the most appropriate specimen type for a given situation.

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