

Papers

Evaluation of a new amplified enzyme immunoassay (EIA) for the detection of *Chlamydia trachomatis* in male urine, female endocervical swab, and patient obtained vaginal swab specimens

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Abstract

Aims—To compare the performance of a new generation dual amplified enzyme immunoassay (EIA) with a molecular method for the diagnosis of *Chlamydia trachomatis*, using a range of urogenital samples, and to assess the reliability of testing self collected vaginal specimens compared with clinician collected vaginal specimens.

Methods—Two population groups were tested. For the first population group, first void urine samples were collected from 193 male patients with urethritis, and endocervical swabs were collected from 187 high risk commercial sex workers. All urine and endocervical specimens were tested by a conventional assay (IDEIA chlamydia), a new generation amplified immunoassay (IDEIA PCE chlamydia), and the Amplicor polymerase chain reaction (PCR). Discrepant results obtained among the three sample types were confirmed using a nested PCR test with a different plasmid target region. For the second population group, four swab specimens, including one patient obtained vaginal swab, two clinician obtained endocervical swabs, and one clinician obtained vaginal swab, were collected from 91 high risk sex workers. Self collected and clinician collected vaginal swabs were tested by IDEIA PCE chlamydia. Clinician obtained endocervical swabs were assayed by IDEIA PCE chlamydia and Amplicor PCR.

Results—The performance of the IDEIA PCE chlamydia test was comparable to that of the Amplicor PCR test when male urine and female endocervical swab specimens were analysed. The relative sensitivities of IDEIA, IDEIA PCE, and Amplicor PCR on male first void urine specimens were 79.3%, 91.4%, and 100%, respectively. The relative sensitivities of the three tests on female endocervical specimens were 85.0%, 95.0%, and 100%, respectively. The positivity rates for pa-

tient collected vaginal specimens and clinician collected vaginal specimens by IDEIA PCE were 25.2% and 23.1%, respectively, whereas those for clinician collected endocervical swabs by PCR and IDEIA PCE were both 27.5%.

Conclusions—IDEIA PCE chlamydia is a lower cost but sensitive alternative test to PCR for testing male urine samples and female endocervical swabs. In addition, self collected or clinician collected vaginal specimens tested by IDEIA PCE chlamydia are a reliable alternative to analysing endocervical specimens.

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Keywords: *Chlamydia trachomatis*; enzyme immunoassay; clinical specimens

Chlamydia trachomatis infection is the most common bacterial sexually transmitted disease (STD) in Japan, and routine screening of high risk patients and selected female populations is widely performed.¹ Recently, nucleic acid amplification techniques, such as the polymerase chain reaction (PCR) and ligase chain reaction (LCR) have become available, with the potential to offer improved sensitivity for diagnosing *C trachomatis* infections. These DNA amplification methods are reported to be more sensitive than cell culture techniques or conventional antigen detection tests, such as enzyme immunoassay (EIA).^{2–4} However, despite the advent of DNA amplification technology, EIA tests are still widely used for the diagnosis of *C trachomatis* in Japan. Although molecular amplification analysis is increasingly used for confirmation testing, its use as a routine screening test for *C trachomatis* is limited by the high cost for each test compared with current routine methods.⁵ This can be offset if samples are pooled before testing,⁶ or on the basis of the calculation of longer term health care cost saving.⁷ It has been suggested that wider screening or universal screening of female populations using molecular amplification techniques will reduce the incidence of

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longer term complications of *C trachomatis* infection.⁸

For wider screening of the female population a non-invasive alternative to endocervical swabs is required. Female urine specimens have been assessed,⁹ but recent reports have indicated inadequate sensitivity compared with testing endocervical swabs because infection mainly occurs in the cervix and less frequently involves the urethra,¹⁰ and because of inhibitors present in urine.¹¹ Recently, DNA amplification testing of vaginal specimens obtained by clinicians or patients themselves has been reported to have comparable sensitivity to that of testing endocervical specimens.¹²⁻¹⁵

The advent of a new generation of sensitive immunoassays for detecting chlamydia lipopolysaccharide (LPS) might offer an opportunity for a lower cost test for wider screening programmes, while providing comparable sensitivity to molecular amplification methods.

The IDEIA PCE chlamydia test is a new, qualitative dual amplified EIA for the detection of chlamydial specific LPS antigens. The principle of IDEIA PCE chlamydia is based on the use of dual label and signal amplification. In addition to the signal amplification system used in an established conventional EIA test (IDEIA chlamydia)¹⁶, the new technology incorporates the use of a polymer conjugate enhanced (PCE) system consisting of a dextran backbone to which multiple anti-chlamydia LPS monoclonal antibody molecules and alkaline phosphatase molecules are bound. It has been reported that the use of polymer conjugates can increase assay sensitivity approximately 40-fold compared with conventional methods.¹⁷ In a previous study we assessed the reliability of the IDEIA PCE chlamydia test when applied to genital swabs collected from high risk sex workers.¹⁸ In assessing vaginal specimens we took the opportunity to compare clinician obtained vaginal swabs with patient obtained vaginal swabs as an indicator of the value of this sample type for community screening for *C trachomatis* infection.

Materials and methods

STUDY POPULATION

Samples were collected from two population groups. The first population of 380 comprised 193 men with symptoms of urethritis and 187 high risk female commercial sex workers, who visited two STD clinics in Fukuoka, Japan, from April to December 1997. The second population group consisted of 91 high risk female commercial sex workers who attended one of the STD clinics from January to March 1998.

SAMPLE COLLECTION

For the first population group, first void urine (20–30 ml) was collected from male patients into sterile screw cap tubes and transported to the laboratory, where it was divided into three aliquots. The first aliquot (10 ml) was used for the IDEIA chlamydia and IDEIA PCE chlamydia (a newly improved EIA kit) tests (Dako, Ely, Cambridgeshire, UK); the second aliquot (8 ml) was used for the Amplicor PCR assay

(Roche Molecular Systems, Branchburg, New Jersey, USA). The final aliquot was stored at –20°C for further evaluation of discrepant results.

For each woman, two endocervical specimens were obtained with a speculum by inserting a swab into the endocervix. Before sampling, the endocervix was cleaned with a swab to remove excess mucous. The swab was rotated several times before withdrawal. The first swab was placed into an Amplicor transport tube and the second into IDEIA transport medium, as provided with each kit. IDEIA chlamydia and Amplicor PCR specimen collection kits for swabs were used in accordance with each manufacturer's recommendation.

For assessment of vaginal specimens (second population group), four swab specimens, including one patient obtained vaginal swab, two clinician obtained endocervical swabs, and one clinician obtained vaginal swab, were collected from each woman. Initially, each woman was asked to obtain a vaginal swab specimen by inserting the swab about 3–5 cm into the vagina, rotating it several times, and removing it. The swab was placed into an IDEIA transport tube by a clinician. Then, a vaginal swab and two endocervical swab specimens (in that order) were obtained by a clinician using a speculum. The clinician obtained vaginal swab was placed into IDEIA transport medium. Of the two endocervical swabs, the first swab was placed into Amplicor transport medium and the second into IDEIA transport medium.

TESTING OF SAMPLES

In the first study group, first void urine and endocervical specimens were processed and tested by the IDEIA chlamydia test, the IDEIA PCE chlamydia test, and the Amplicor PCR assay, according to each manufacturer's instructions. For the second population group, patient obtained and clinician obtained vaginal swab specimens were assayed by the IDEIA PCE chlamydia kit, and endocervical swab specimens were assayed by the IDEIA PCE chlamydia kit and the Amplicor PCR test. Urine and endocervical swab specimens were stored at 2–8°C for up to three days until processed and measured with the Amplicor PCR test as described in detail in our previous study.² The results were interpreted according to instructions and quality control criteria provided by the manufacturer.

RESOLUTION OF DISCREPANCIES AND CONFIRMATORY TESTING

For evaluation of urine and endocervical specimens (first population group), a specimen was considered to be positive for *C trachomatis* infection if the IDEIA PCE test and the Amplicor PCR assay gave positive results. When there was a discrepancy between the IDEIA PCE and PCR results, nested PCR with a different plasmid target region from that of the Amplicor PCR test was performed as a confirmatory test. The first PCR amplification was performed using primers CT2 and CT5, as described previously.¹⁹ The reaction product

Table 1 Results of the detection of *Chlamydia trachomatis* in male first void urine and female endocervical swab specimens by IDEIA, IDEIA PCE, and AmpliCor PCR

Sample source	No. of specimens	Procedures			Final results	
		IDEIA	IDEIA PCE	AmpliCor PCR	Positive	Negative
Male first void urine	135	-	-	-	0	135
	46	+	+	+	46	0
	7	-	+	+	7	0
	5*	-	-	+	5	0
	Total no. (n = 193)	46	53	58	58	135
Female endocervical swab	146	-	-	-	0	146
	34	+	+	+	34	0
	4	-	+	+	4	0
	3†	-	-	+	2	1
	Total no. (n = 187)	34	38	41	40	147

Total no. refers to the number of confirmed positive cases by each assay.

*All five were positive by confirmatory nested PCR.

†Of three specimens, two were positive and one negative by confirmatory nested PCR.

IDEIA, enzyme immunoassay; PCE, polymer conjugate enhanced; PCR, polymerase chain reaction.

was then amplified for a second time using primers CT7 (5'-GGATTTATCGGAAACC TTGA-3') and CT8 (5'-CTTTCAATGG AATAGCGGGT-3'), with all other conditions remaining the same.¹⁹ The amplified product (10 µl) was analysed by electrophoresis on a 2% agarose gel. If a specimen was positive using the supplementary testing, combined with one other positive test result (IDEIA PCE or AmpliCor PCR), the sample was confirmed as being positive for *C trachomatis*. After resolution of the discrepancies, relative sensitivity and specificity, positive and negative predictive values, and 95% confidence intervals were calculated. Statistical analysis of the data was also performed using the Pearson χ^2 test. A p value < 0.05 was considered significant.

For the evaluation of vaginal specimens (second population group), a woman was considered to be infected with *C trachomatis* if the AmpliCor PCR or IDEIA PCE chlamydia test was positive for the clinician obtained endocervical swab. A woman who had a positive vaginal swab but negative endocervical swabs was considered to be positive if she was confirmed IDEIA PCE chlamydia positive using the IDEIA blocking test for vaginal swabs. The IDEIA blocking test was performed and results interpreted according to information provided by the manufacturer.

Results

URINE AND ENDOCERVICAL SPECIMENS

The results of the detection of *C trachomatis* in male and female specimens using IDEIA PCE

were compared with those obtained by IDEIA and AmpliCor PCR (table 1). Of 193 male first void urine specimens tested, 135 were negative and 46 were positive by IDEIA, IDEIA PCE, and AmpliCor PCR. Twelve discrepant results were obtained. Of the 12 specimens, seven were positive according to IDEIA PCE and AmpliCor PCR. Thus, these seven men were considered to be positive for *C trachomatis*. The remaining five specimens were confirmed as being positive using the nested PCR assay. After resolution of discrepancies, of the 193 male urine specimens tested, 58 (30.1%) were positive for *C trachomatis* and 135 (69.9%) were negative. Of 187 female endocervical swab specimens tested, 146 were negative and 34 were positive by IDEIA, IDEIA PCE, and AmpliCor PCR. Seven discrepant results were obtained. Of the seven specimens, four were positive by IDEIA PCE and AmpliCor PCR. Thus, these four specimens were considered to be positive for *C trachomatis* infection. Of the remaining three specimens, two were confirmed as being positive and one negative using nested PCR. After resolution of discrepancies, of the 187 endocervical specimens tested, 40 (21.4%) were considered to be positive for *C trachomatis* and 147 (78.6%) were negative. The relative sensitivity and specificity, 95% confidence intervals, and predictive values were then calculated according these results (table 2). The relative sensitivities of IDEIA, IDEIA PCE, and AmpliCor PCR on male first void urine specimens were 79.3%, 91.4%, and 100%, respectively. The relative sensitivities of IDEIA, IDEIA PCE, and AmpliCor PCR on female endocervical swab specimens were 85.0%, 95.0%, and 100%, respectively. There were no statistical differences between the sensitivities of the AmpliCor PCR assay, the IDEIA PCE chlamydia test, and the IDEIA chlamydia test.

VAGINAL SPECIMENS

Of 91 women tested, 64 were negative for all four sample types collected and 20 were positive for all four sample types collected (two clinician obtained endocervical swabs for AmpliCor PCR and IDEIA PCE, and one clinician obtained and one patient obtained vaginal swab for IDEIA PCE) (table 3). There were seven discrepancies among the sample types collected. Of the seven women, six were confirmed to be infected with *C trachomatis* because the AmpliCor PCR assay or the IDEIA PCE test was positive for the clinician obtained

Table 2 Performance of IDEIA, IDEIA PCE, and AmpliCor PCR for the detection of *Chlamydia trachomatis* in male first void urine and female endocervical swab specimens

Sample source and procedure	Prevalence (%)	Relative sensitivity (%) (95% CI)	Relative specificity (%) (95% CI)	Predictive value (%)	
				Positive (95% CI)	Negative (95% CI)
Male first void urine					
IDEIA	23.8 (46/193)	79.3 (74.8 to 88.8)	100 (97.3 to 100)	100 (92.3 to 100)	91.8 (86.2 to 95.7)
IDEIA PCE	27.5 (53/193)	91.4 (81.1 to 94.2)	100 (97.3 to 100)	100 (93.3 to 100)	96.4 (91.9 to 98.8)
AmpliCor PCR	30.1 (58/193)	100 (95.9 to 100)	100 (97.3 to 100)	100 (93.8 to 100)	100 (97.3 to 100)
Female endocervical swab					
IDEIA	18.2 (34/187)	85.0 (70.1 to 94.3)	100 (97.5 to 100)	100 (89.7 to 100)	96.7 (92.5 to 98.9)
IDEIA PCE	20.3 (38/187)	95.0 (83.1 to 99.4)	100 (97.5 to 100)	100 (90.8 to 100)	98.7 (95.2 to 99.8)
AmpliCor PCR	21.9 (41/187)	100 (91.3 to 100)	99.3 (96.3 to 100)	97.6 (87.1 to 99.9)	100 (97.5 to 100)

CI, confidence interval; IDEIA, enzyme immunoassay; PCE, polymer conjugate enhanced; PCR, polymerase chain reaction.

Table 3 Results of the detection of *Chlamydia trachomatis* in patient obtained vaginal swab (VS) and clinician obtained endocervical swab (ES) and vaginal swab specimens

No. of specimens	IDEIA PCE				Final results	
	Patient obtained VS	Clinician obtained VS	Clinician obtained ES	Amplicor PCR Clinician obtained ES	Positive	Negative
64	–	–	–	–	0	64
20	+	+	+	+	20	0
3	–	–	+	+	3	0
1	+	+	+	–	1	0
1	+	–	+	+	1	0
1	–	–	–	+	1	0
1*	+	–	–	–	1	0
Total no. (n = 91)	23	21	25	25	27	64

Total no. refers to the number of confirmed positive cases by each assay.

*This specimen was positive by the confirmatory IDEIA PCE blocking test.

IDEIA PCE, enzyme immunoassay polymer conjugate enhanced; PCR, polymerase chain reaction.

endocervical specimens. The remaining woman was confirmed to be positive by the IDEIA PCE blocking assay using the patient obtained vaginal swab. After resolution of discrepancies, of the 91 women tested, 27 (29.8%) were found to be infected with *C trachomatis* and 64 (70.2%) were found not to be infected. The positivity rates for patient collected vaginal specimens and clinician collected vaginal specimens were 25.2% (23 of 91) and 23.1% (21 of 91), respectively; those for clinician collected endocervical swabs by PCR and IDEIA PCE were similar—both 27.5% (25 of 91). There was no difference between testing self collected or clinician collected specimens and between testing vaginal specimens and endocervical swabs.

Discussion

In Japan, commercial PCR or LCR assay kits are available as routine tests for the detection of *C trachomatis*. However, these DNA amplification tests are extremely costly⁷ compared with conventional EIAs for antigen detection. Furthermore, these tests require specialised facilities to reduce Amplicor PCR contaminants. More recently, a new generation dual amplified immunoassay IDEIA PCE chlamydia has become available, which has been shown to be diagnostically reliable when applied to genital swabs.¹⁸ The IDEIA PCE chlamydia test is 2.5 to five times more sensitive for the detection of *C trachomatis* elementary bodies than the conventional EIA test (IDEIA).²⁰ Currently, several studies have shown that analysis of urine specimens using DNA amplification methods is a possible alternative to the analysis of endocervical specimens for chlamydia diagnosis in women.^{21–22} However, the sensitivity was lower when using female urine specimens than when endocervical specimens were used.^{10–21–22} The reason for this reduced sensitivity is that most women are infected with *C trachomatis* at the endocervix, a site remote from the urethra. Therefore, urine samples might not be suitable for the detection of endocervical infection. Moreover, the handling and laboratory processing of urine specimens is more difficult compared with endocervical or vaginal swab specimens. Recent publications have also shown that DNA amplification

testing for chlamydia with patient obtained vaginal swabs is as sensitive as endocervical testing.^{13–15} Patient obtained vaginal swabs seems to be a more suitable and less invasive method for the screening for *C trachomatis* than clinician obtained endocervical or vaginal specimens. To our knowledge, reports on *C trachomatis* detection in patient obtained vaginal swab specimens using an EIA test are very rare.

In the first part of our study, we compared the performance of IDEIA PCE with that of the IDEIA test and the commercially available PCR assay (Amplicor) in male first void urine and female endocervical samples. The positivity rates for IDEIA PCE on male first void urine and female endocervical swab specimens (urine, 27.5%; endocervical swab, 20.3%) were higher than those for IDEIA (urine, 23.8%; endocervical swab, 18.2%), and comparable with the Amplicor PCR assay (urine, 30.1%; endocervical swab, 21.9%). However, the results obtained in our study might not reflect the true clinical sensitivity of each test because samples were not tested by culture, and no allowance was made for amplification inhibitors, which might be present in some samples. Moreover, the discrepant analysis procedure used was only applied to the discrepant samples, and not the whole population tested, and this might have introduced some bias into the data analysis.²³ Other studies have reported EIAs to be less sensitive than amplification tests.^{2–4} These EIAs are generally based on passive capture of chlamydia LPS and use conventional signal generation systems. The incorporation of dual immunoassay amplification technology into the IDEIA PCE test might explain why we obtained a comparable positivity rate to PCR. Moreover, the cost for each IDEIA PCE test is similar to IDEIA, but much lower than the Amplicor PCR assay. However, a large study might be required to assess the true clinical performance and value of the IDEIA PCE kit because the population size tested and number of positive samples in our study are not sufficient.

In the second part of our study, we evaluated the clinical importance of patient obtained vaginal swab specimens using a new EIA kit. The results demonstrated that testing self collected vaginal specimens in a Japanese population was as reliable as testing clinician collected specimens, and that testing vaginal specimens by IDEIA PCE chlamydia was an acceptable alternative to testing endocervical swabs by IDEIA PCE chlamydia or Amplicor PCR. The agreement between the positivity rates obtained for vaginal swabs and endocervical swabs was closer than has been reported for studies comparing urine specimens with endocervical swabs.^{10–21}

The prevalence rate of *C trachomatis* in the commercial sex workers tested was approximately 20–30%. This prevalence rate among these women is much higher than that in the general Japanese female population (approximately 5%).²⁴ Although the population tested was mainly asymptomatic, the prevalence was high because of the occupation of the

population tested. In our city, female commercial sex workers are a major reservoir of STDs. To prevent the spread of *C trachomatis* infection to the general population, continuous close monitoring of *C trachomatis* infection among commercial sex workers is necessary. In this regard, patient obtained vaginal swabs using the IDEIA PCE test would be useful for the screening of *C trachomatis* among commercial sex workers and offers the potential for cost effective, reliable, and less invasive screening of high risk/prevalence female populations. However, our results might not be applicable to lower prevalence populations, such as those seen in family planning clinics, because the carriage of *C trachomatis* will be lower. Furthermore, a large study is required to evaluate the true clinical usefulness of patient vaginal swabs as an alternative to endocervical swabs.

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