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## Chlamydia Trachomatis Detection in Cervical PreservCyt Specimens From an Irish Urban Female Population

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## ***Chlamydia trachomatis* detection in cervical PreservCyt specimens from an Irish urban female population**

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### ***Chlamydia trachomatis* detection in cervical PreservCyt specimens from an Irish urban female population**

**Objective:** The aim of this study was to determine the prevalence of cervical *Chlamydia trachomatis* infection by polymerase chain reaction (PCR) in urban women undergoing routine cervical cytological screening and to investigate the relationship with age, cytology, smoking status and concurrent human papillomavirus (HPV) infection.

**Methods:** A total of 996 women (age range 16–69 years) attending general practitioners for routine liquid-based cervical smear screening in the Dublin area were recruited in the study of prevalence of *C. trachomatis*. Informed consent was obtained and liquid-based cytology (LBC) specimens were sent for cytological screening. DNA was extracted from residual LBC and tested for *C. trachomatis* by PCR using the highly sensitive *C. trachomatis* plasmid (CTP) primers and for HPV infection using the MY09/11 primers directed to the HPV L1 gene in a multiplex format.

**Results:** The overall prevalence of *C. trachomatis* was 5.4%. Prevalence was highest in the <25 years age group (10%). Coinfection with HPV and *C. trachomatis* occurred in 1% of the screening population. A higher rate of smoking was observed in women positive for *C. trachomatis*, HPV infections or those with abnormal cervical cytology. *Chlamydia trachomatis* infection was not associated with abnormal cytology.

**Conclusions:** Women (5.4%) presenting for routine cervical screening are infected with *C. trachomatis*. Opportunistic screening for *C. trachomatis* from PreservCyt sample taken at the time of cervical cytological screening may be a possible strategy to screen for *C. trachomatis* in the Irish female population.

**Keywords:** *Chlamydia trachomatis*, PreservCyt, cervical cytology, human papillomavirus, smoking, Irish

### **Introduction**

*Chlamydia trachomatis* is the most common bacterial sexually transmitted infection (STI) worldwide with approximately 90 million cases occurring annually.<sup>1</sup> *Chlamydia trachomatis* causes a variety of disease states ranging from asymptomatic infections, cervicitis and pelvic inflammatory disease to ectopic pregnancies and tubal infertility with each successive round of infection increasing the risk of serious

sequelae.<sup>2</sup> High-risk human papillomavirus (HPV) infection of the cervix is necessary for the development of preneoplastic cervical lesions, which may be detected on Pap smear.<sup>3</sup> Cigarette smoking and *C. trachomatis* infections are now considered independent risk factors for the development of cervical cancer.<sup>4–6</sup>

In Ireland, the incidence of *C. trachomatis* infections is rising each year, with 2803 cases reported during 2004.<sup>7</sup> Consequently, the need for *C. trachomatis* screening in Ireland is under review.<sup>8</sup> Currently in the USA, Centre for Disease Control and Prevention recommends that women <25 years, women with multiple sexual partners, women having had a change in partner, women who have symptoms suggestive of chlamydial infection and those who have had a

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previous STI are screened at regular intervals.<sup>9</sup> These recommendations have been translated into active screening programmes across all states, with well-documented evidence of a reduction in prevalence in areas where intervention has been in place for a number of years.<sup>10</sup> Similarly, in Sweden, a national *C. trachomatis* screening programme implemented in the 1980s has been associated with a dramatic reduction in incidence of *C. trachomatis* and its adverse sequelae.<sup>11</sup>

Commercial nucleic acid-based *C. trachomatis* detection methods such as the AmpliCor<sup>®</sup> CT/NG Test (RocheMolecular Systems, Branchburg, NJ, USA), the Digene hybrid capture (HCII; Digene, Gaithersburg, MD, USA) and the APTIMA Combo-2 assay (Genprobe Inc., San Diego, CA, USA) demonstrate both high sensitivities and specificities.<sup>12,13</sup> They commonly target the *C. trachomatis* multicopy plasmid genes and are routinely performed on cervical swabs or urines.<sup>12,13</sup> The use of molecular methods for the detection of high-risk HPV DNA and mRNA from PreservCyt cervical specimens has substantial potential and molecular testing for HPV has been proposed as an adjunct to cervical cytology in screening algorithms.<sup>14,15</sup> Many studies have demonstrated the feasibility of screening PreservCyt specimens for detecting infections in the genital tract other than HPV<sup>16–18</sup> and other studies have reported on the stability of nucleic acids in PreservCyt.<sup>19,20</sup>

The aim of this study was to determine the prevalence of *C. trachomatis* and HPV infections in Irish women attending their general practitioner (GP) for a cervical smear test. *C. trachomatis* infections were analysed based on age, smoking status, cervical cytology and coinfection with HPV.

## Methods

### *Study cohort*

The population consisted of 996 women who attended one of nine participating GPs in Dublin city and suburban areas for cervical smear testing over a period of 14 months between December 2003 and February 2005. Women were recruited to the study regardless of previous history or symptoms of disease. Women were invited to participate in the study of prevalence of *C. trachomatis* by the GP on receipt and understanding of an information leaflet and on completion of a consent form. Details of current cigarette smoking status, age and cytological diagnosis were obtained.

The study was anonymized and no patient identifiers were recorded.

### *Ethical approval*

Ethical approval was obtained for the study from the St. James' Hospital Ethics Committee Review Board in August 2003.

### *Specimen collection and processing*

Cervical specimens were taken and placed in a vial of PreservCyt (Cytyc Corporation, Marlborough, MA, USA) medium and transported to St. James' Cytology Laboratory where a cervical smear was prepared using the ThinPrep (Cytyc Corporation, USA) processor. Residual specimens were then kept at room temperature until DNA was extracted as described previously.<sup>21</sup> Briefly, PreservCyt specimen (4 ml) was vortexed vigorously, then centrifuged at 3000 *g* and the pellet was washed twice with TE buffer (10 mM Tris, 1 mM ethylenediaminetetraacetic acid, pH 8.0). Cell pellets were resuspended in TE buffer (200 µl) and DNA was extracted using the QIAamp DNA Mini Kit (Qiagen Ltd, Crawley, UK) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed using the CTP primers for detection of the *C. trachomatis* cryptic plasmid and MY09/11 primers for the detection of high and low-risk HPV in a multiplex format as described previously.<sup>22</sup> The multiplex PCR included primers for amplification of human β-globin to ensure quality of the nucleic acid extraction.

### *Statistical analysis*

Statistical data were analysed using SPSS version 11.0 software. Pearson Chi-square tests were performed to compare prevalence of *C. trachomatis* with age, smoking status, abnormal cytology and HPV coinfection.

## Results

### *Study population*

The age of the study population ranged from 16 to 72 years. The average age of women presenting for routine cervical screening was 35 years. Of the population studied, 187/996 (19%) were <25 years, 401/996 (40%) were between the ages of 25 and 35 years and 408/996 (41%) >35 years (Table 1).

**Table 1.** Prevalence of *Chlamydia trachomatis* in cervical PreservCyt specimens and age ( $n = 996$ )

<i>C. trachomatis</i>	Age		
	<25	25–35	>35
Positive	18 (10)	20 (5)	16 (4)
Negative	169 (90)	381 (95)	392 (96)
Total	187	401	408

Values are represented as  $n$  (%).

#### Prevalence of *C. trachomatis*

The overall prevalence of *C. trachomatis* was 5.4%. Prevalence was 10% (18/187) in the age group of <25 years, 5% (20/401) in the 25–35 years and 4% (16/408) in the >35 years. Thirty-three per cent (18/54) of all *C. trachomatis* infections were in the <25 years age group, 37% (20/54) in the 25–35 years age group and 30% (16/54) in the >35 years age group (Table 1). Cumulatively 70% (38/54) of *C. trachomatis* infections occurred in the <35 years age group. The trend of decreasing prevalence of *C. trachomatis* with age was highly significant ( $P < 0.0001$ ).

#### Coinfection with *C. trachomatis* and HPV

Of the 54 *C. trachomatis*-infected specimens, 11 (20.4%) also contained HPV. The overall coinfection rate within the population studied was 1%. The average age of women infected with both organisms was 31 years with 8/11 concomitant infections occurring in women under 35 years of age.

#### *C. trachomatis* and cervical cytology

Of the 54 *C. trachomatis*-positive samples, 50 (92.6%) had normal cytology, 2 (3.7%) had borderline cytology and 2 (3.7%) had cervical intraepithelial neoplasia grade I (CIN-I) lesions. *C. trachomatis* infection was not statistically associated with abnormal cytology.

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#### Smoking and cervical cytology

Details of tobacco smoking were obtained for 706 of the 997 women in the study. Overall, 191/706 (27.1%) of individuals admitted to smoking on a daily basis. However, no information on number of cigarettes or duration of smoking was available for this study. Of the 191 smokers, 19.4% had some degree of abnormal cytology, i.e. evidence of either borderline cytology or CIN lesions versus 7.4% of non-smokers (Table 2). Smoking was more common in women with abnormal cytology ( $P < 0.0001$ ). The percentage of women within each category of abnormal cytology was higher for the smokers than the non-smokers (Table 2).

#### Smoking and prevalence of *C. trachomatis* and HPV infections

Of the women who smoked 46/191 (24%) had HPV infections and 15/191 (8%) had *C. trachomatis* infections. Of the non-smokers, 80/515 (16%) had HPV infections and 23/515 (4%) had *C. trachomatis* infections (Table 3). Smoking was statistically associated with both HPV and *C. trachomatis* infections ( $P = 0.008$  and  $P < 0.001$ ). Four of 191 (2.1%) smokers were coinfecting with HPV and *C. trachomatis* versus 2/515 (0.4%) non-smokers (Table 3).

## Discussion

Screening for *C. trachomatis* may contribute to the prevention of pelvic inflammatory disease and reduce the cost of reproductive health problems,

**Table 2.** Smoking status and cytology result ( $n = 706$ )

Smoking status	Cervical cytology					
	Normal	Abnormal*	Borderline <sup>†</sup>	CIN-I	CIN-II	CIN-III
Smoker ( $n = 191$ )	154 (80.6)	37 (19.4)	16 (8.4)	15 (7.8)	3 (1.6)	3 (1.6)
Non-smoker ( $n = 515$ )	477 (92.6)	38 (7.4)	15 (2.9)	16 (3.1)	6 (1.2)	1 (0.2)
Total ( $n = 706$ )	631	75	31	31	9	4

Values are represented as  $n$  (%).

\*Classified as having either borderline cytology or cervical intraepithelial neoplasia (CIN).

<sup>†</sup>Borderline nuclear changes, including atypical glandular and squamous cells of undetermined significance.

Smoking status	HPV		<i>C. trachomatis</i>		Coinfection	
	Negative	Positive	Negative	Positive	Negative	Positive
Smoker ( <i>n</i> = 191)	145 (76)	46 (24)	176 (92)	15 (8)	187 (98)	4 (2)
Non-smoker ( <i>n</i> = 515)	435 (84)	80 (16)	492 (96)	23 (4)	513 (99.6)	2 (0.4)
Total ( <i>n</i> = 706)	580	126	668	38	700	6

Values are represented as *n* (%).

HPV, human papillomavirus.

**Table 3.** Smoking status, HPV and *Chlamydia trachomatis* infection and coinfection (*n* = 706)

such as ectopic pregnancies and infertility. Before any effective screening programme is introduced into a population, it is necessary to determine the expected prevalence rate and identify those groups who should be targeted. Ireland is currently examining the need for a *C. trachomatis* screening programme. The aim of this study was to determine the prevalence of *C. trachomatis* in an urban female population undergoing routine opportunistic cervical screening.

In this study, a multiplex PCR was performed to screen samples simultaneously for HPV and *C. trachomatis*. The sensitivity and specificity of this assay was determined previously with respect to the commercially available HCII assay for HPV detection and the ligase chain reaction assay (LCx; Abbott Laboratories, Abbott Park, IL, USA) for *C. trachomatis* detection (results not shown). The sensitivity and specificity of the multiplex for the detection of HPV with respect to the HCII assay were 95% and 100%, respectively. The sensitivity and specificity of the multiplex for the detection of *C. trachomatis* were 100% with respect to the commercial LCx assay. In our study, an overall prevalence of *C. trachomatis* of 5.4% was determined with a prevalence of 10% in the <25 years. It is widely known that the prevalence of *C. trachomatis* depends upon the setting, context and country studied. In 2002, the European Union BioMed Concerted Action Group undertook a systematic review of over 300 studies of prevalence of *C. trachomatis* among European women. The mode was 6% for women seeking contraception and 4% for women having cervical smears.<sup>23</sup> Our finding of 5.4% in women having cervical smears correlates well with the European average. In a systematic literature review of cost-effectiveness studies conducted between 1990 and 2000, screening for *C. trachomatis* was cost-effective at prevalences of 3.1–10% and cost saving at prevalences of >1.1% if age was used as a selection factor and DNA-based tests on urine used.<sup>24</sup> Based on these

figures opportunistic screening in the Irish population would be cost-effective particularly for the <25 years.

STI surveillance in Ireland is mostly genitourinary medicine clinic based, with few incidence reports made from primary care settings.<sup>7</sup> HPV and *C. trachomatis* infections are among the most common cases of STI reported in Ireland. In 2004, the three most commonly reported STIs were ano-genital warts (*n* = 4174), *C. trachomatis* (*n* = 2803) and non-specific urethritis (*n* = 2746).<sup>7</sup> Few studies have investigated the prevalence of *C. trachomatis* in the Republic of Ireland. In 2004, a study was conducted in the mid-western region to determine the prevalence of *C. trachomatis* in men. Of 562 men attending orthopaedic clinics and university sports facilities, 5.9% were positive for *C. trachomatis*.<sup>25</sup> Recently a study was conducted to determine the prevalence of *C. trachomatis* in women attending a maternity hospital for antenatal, fertility and family planning services. A prevalence of *C. trachomatis* of 3.7% was found in urine samples.<sup>26</sup> Testing of urine samples using nucleic acid-based techniques has often been criticized due to the presence of amplification inhibitors in urine.<sup>27–29</sup> In our study, DNA was extracted from residual cells in PreservCyt medium following routine cervical smear testing from which all samples amplified for the internal control and no amplification inhibition was observed. Our prevalence of 5.4% may be a truer estimate of *C. trachomatis* infections in the Irish female urban population regardless of parity or fertility status.

Previous studies have demonstrated a decrease in prevalence of *C. trachomatis* with age.<sup>30,31</sup> This trend was observed in our study with incidence reducing from 10% in the <25 years age group to 5% in the 25–35 years and 4% in the >35 years. The incidence of *C. trachomatis* infections was highest in the <25 years age group; however, this group is not commonly targeted for cervical screening. Since the majority of *C. trachomatis* infections are asymptomatic,



testing of cervical PreservCyt samples may be a cost effective strategy for the screening of sexually active women.

In our study, those who smoked had a higher incidence of *C. trachomatis* and HPV infections than non-smokers. However, the strength of this association is limited by the lack of detailed information on number of cigarettes smoked per day and the duration of smoking. The higher incidence may be as a result of lifestyle factors linking high-risk sexual behaviour to unhealthy lifestyle choices. Other studies have also demonstrated a positive association of HPV and *C. trachomatis* infection with current smoking.<sup>32,33</sup>

*Chlamydia trachomatis* is now considered an independent risk factor for the development of cervical cancer.<sup>34</sup> A recent study on colposcopy patients reporting a prevalence of 3.4%, suggested that routine screening for *C. trachomatis* be carried out in colposcopy clinics.<sup>35</sup> In our study, 20.4% of *C. trachomatis* infected samples were coinfecting by HPV; however, no association was seen between *C. trachomatis* infection and abnormal cytology. A recent study in Argentina found that prevalence of *C. trachomatis* was higher in HPV-infected cohorts.<sup>36</sup> *Chlamydia trachomatis*-infected cohorts may also overlap with those infected by other STIs and identify those at increased risk of cervical neoplasia.

Numerous studies conducted in other countries have evaluated and advocated opportunistic *C. trachomatis* screening approaches in primary health care settings.<sup>37–39</sup> While urine testing has been the mainstay in screening for *C. trachomatis*, liquid-based cytology affords the simultaneous evaluation of cytology, HPV and *C. trachomatis* from a single sample. Opportunistic screening for *C. trachomatis* at the time of cervical screening would not only identify women at risk for adverse reproductive complications but taken together with cytology result, HPV status, smoking status and other infecting STI identify those at higher risk for development of cervical neoplasia.

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