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## Endocervical Gram stain smears and their usefulness in the diagnosis of *Chlamydia trachomatis*

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**Objective:** To evaluate the usefulness of endocervical Gram stain smears in the diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in a female population attending a STD clinic.

**Methods:** 494 females attending a STD clinic and undergoing a speculum examination had endocervical specimens submitted for *C trachomatis* culture, direct fluorescent antibody testing (DFA), and *N gonorrhoeae* culture. Endocervical smears were also collected for Gram stain. The number of polymorphonuclear leucocytes (PMN) per high power field (HPF), presence of bacteria, yeast, red blood cells, and clue cells were recorded. Clinical signs of cervicitis were also documented.

**Results:** *N gonorrhoeae* was isolated from one subject who was co-infected with *C trachomatis* and no further analysis was done regarding *N gonorrhoeae*. Analysis was performed on 220 participants. The prevalence of *C trachomatis* infection was 13%. Of the Gram smears collected, 55% were inadequate owing to the presence of vaginal contamination. There were an equal number of *C trachomatis* isolates in patients with  $\leq 10$  PMN/HPF (48%) and  $>10$  PMN/HPF (52%). Endocervical mucopus and erythema were statistically significant for the presence of *C trachomatis* ( $p < 0.001$  and  $0.02$  respectively). The presence of any signs of cervicitis—that is, mucopus, friability, erythema, and ectropion together with  $>10$  PMN/HPF was statistically significant for the presence of *C trachomatis*.

**Conclusion:** The use of endocervical Gram smear results together with clinical information can be used to identify high risk women for *C trachomatis* infection.

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Keywords: endocervical Gram stain; polymorphonuclear leucocytes; *Chlamydia trachomatis*

### Introduction

Genital infection by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* cause considerable reproductive morbidity in women. Unfortunately, a large proportion of these women are asymptomatic and their infection remains undetected.<sup>1–6</sup> In light of this situation many women attending their general practitioner are not routinely screened for these two pathogens. Additionally, laboratory culture for *C trachomatis* is costly, time consuming, requires expertise, and extensive laboratory supplies.<sup>7</sup> However, cell culture may be replaced as the gold standard by other diagnostic methods such as nucleic acid amplification or direct fluorescent antibody testing (DFA). Endocervical Gram stain smears taken during gynaecological examination are an inexpensive, relatively easy procedure to perform and interpret. If specific endocervical changes could be related to the identification of *C trachomatis* and *N gonorrhoeae*, Gram stains would be useful as a screening tool to predict infection by these two micro-organisms.

In a previously published study 214 women attending an STD clinic who were at increased risk of *C trachomatis* infection, underwent endocervical examination and Gram stain interpretation.<sup>8</sup> From 163 valid smears, women with 10 or more polymorphonuclear cells (PMN) per high power field (HPF) were found to be twice as likely to have positive endocervical cultures for *C trachomatis* as those with

fewer than 10 PMN/HPF. Additionally, the presence of mucopurulent endocervical discharge was found to be an independent predictor of infection with chlamydia.

Another study enrolled 193 sexually active female adolescents from a non-sexually transmitted disease clinic.<sup>9</sup> It was found that the presence of  $\geq 5$  PMN/HPF on Gram stains of endocervical secretions was a useful screening tool in predicting endocervical chlamydia infection, but it was also race dependent. The incidence of *C trachomatis* was higher among black subjects compared with non-black subjects. The presence of polymorphonuclear cells was a significant indicator associated with *C trachomatis* for black subjects ( $p < 0.001$ ), but not for non-black subjects ( $p < 0.1$ ). The findings of mucopurulent discharge, friability, or erythema oedema of ectopy were not reliable indicators for *C trachomatis*.

In 1984, a study of 100 women attending a clinic for sexually transmitted diseases observed criteria for the clinical diagnosis of mucopurulent cervicitis.<sup>1</sup> Visualisation of yellow mucopurulent endocervical secretions on a white swab and the presence of 10 or more PMN/HPF were correlated with cervical *C trachomatis* infection. It was recommended that these findings should guide the selective use of confirmatory diagnostic tests for *C trachomatis* infection.

A study was performed at a Seattle STD clinic between 1984 and 1986 which assessed

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Table 1 Correlation of the number of endocervical polymorphonuclear cells with *Chlamydia trachomatis* infection

PMN/HPF	<i>C trachomatis</i>		Sensitivity (%)	Specificity (%)	p Value
	Pos n=29 (%)	Neg n=191 (%)			
<5	10 (35)	113 (59)	35	41	0.02
5-10	4 (14)	22 (12)	14	89	NS
11-20	7 (24)	23 (12)	24	88	NS
>20	8 (28)	33 (17)	28	83	NS
>10 PMN	15 (52)	56 (29)	52	71	0.02
≤10 PMN	14 (48)	135 (71)	48	29	—

NS = not significant.

779 women by various algorithms to identify clinical epidemiological correlates of cervical and vaginal infections.<sup>10</sup> Their findings demonstrated microscopy added very little sensitivity for cervical infection while increasing costs.

Finally, a 1998 study testing Canadian practice guidelines for the presumptive diagnosis of chlamydial cervicitis was performed.<sup>2</sup> It demonstrated that a cut-off value of 10 PMN/HPF on a Gram stained cervical smear is not clinically useful in the presumptive diagnosis of chlamydial cervicitis.

This present study was undertaken to further evaluate the usefulness of the endocervical Gram stain along with the clinical picture in the diagnosis of *C trachomatis* and *N gonorrhoeae* infection.

## Methods

### STUDY POPULATION

A total of 494 females, ≤25 years of age, attending the STD clinic in Edmonton, Alberta, were informed of the research study and consent was given before study participation. Females undergoing a speculum examination, who had been sexually active within the past month, were eligible. Potential subjects were excluded if they were menstruating, had had a hysterectomy, or had taken antibiotics within 14 days.

### STUDY DESIGN

At the time of the speculum examination the cervix was evaluated for the presence of mucopurulent endocervical discharge, friability, erythema, and ectropion. Friability defined as bleeding after any swabbing and endocervical discharge defined as any endocervical mucopurulent discharge. The cervix was then cleaned with gauze and two endocervical smears were obtained and air dried. Routine endocervical samples for *C trachomatis* and *N gonorrhoeae* were also collected and submitted for culture. One endocervical smear was Gram stained and the second was used for the DFA test for *C trachomatis*. The Gram stained smear was evaluated for the number of PMN/HPF, presence of bacteria, yeast, red blood cells, and clue cells. Samples that contained more than 100 squamous epithelial cells were rejected because of the probability of vaginal contamination. Five non-adjacent fields were viewed at a magnification of ×1000, with oil immersion microscopy. The number of polymorphonuclear cells was determined, averaged, and categorised as follows: 0 or rare, <5, 5-10, 11-20, 21-30, 31-40, and >40. The Gram

stains were reviewed without knowledge of subject identification, diagnosis, or test results.

Direct fluorescent antibody test (Syva MicroTrak) for *C trachomatis* was performed according to standardised procedures. *C trachomatis* was cultured in cyclohexamide treated McCoy cells grown on circular coverslips in dram shell vials. Fluorescein conjugated monoclonal antibody stain was used to identify chlamydial inclusions. Specimens collected for *N gonorrhoeae* were inoculated onto biplates containing New York City-Henderson medium, incubated, and interpreted according to established protocol.

Through retrospective chart reviews, age, date of last menstrual period, form of contraception, and physical appearance of the cervix were recorded for each participant.

### ETHICAL CONSIDERATION

The study was institutional review board exempt, as no additional procedures were undertaken and it was considered analysis of routine clinical and microbiological data. All subjects were informed of the research study and procedures to be performed. Verbal consent was obtained from all participants.

### STATISTICAL ANALYSIS

Two tailed Fisher's exact test was used to analyse the differences between the groups. p Values ≤0.05 were considered significant.

## Results

A total of 494 participants were recruited between 1997 and 1998; 220 (45%) slides were accepted for analysis. The high prevalence of unacceptable specimens was because of slides with >100 squamous epithelial cells. The mean age of participants was 20.4 years (range 13-25 years).

The prevalence of *C trachomatis* was 13% (29/220). Of the positive *C trachomatis* specimens, three (10%) had discrepant results between the cell culture and DFA test. One specimen was culture positive and DFA negative, two were culture negative and DFA positive, 26 tested positive by both methods, and 191 tested negative for both culture and DFA. The remainder of this analysis is based on the total 29 *C trachomatis* samples.

*N gonorrhoeae* was isolated from only one female and this was a co-infection with *C trachomatis*. Intracellular Gram negative diplococci were not seen on any of the Gram stained smears.

Gram stain results were stratified with respect to density of PMNs to assess the utility of these diagnostic categories in predicting infection with *C trachomatis*.

### CORRELATION BETWEEN *C TRACHOMATIS* AND GRAM STAIN (TABLE 1)

Owing to the small number of positive *C trachomatis* samples, the original PMN breakdown was adjusted to <5, 5-10, 11-20, and >20. With this adjustment, significant data were observed in the <5 PMN group. This same group had the greatest number of positive and negative *C trachomatis* subjects, (p=0.02).

Table 2 Clinical findings as related to identification of *Chlamydia trachomatis*

Clinical findings	<i>C trachomatis</i>		Sensitivity (%)	Specificity (%)	p Value
	Pos n=29 (%)	Neg n=191 (%)			
Mucopus	8 (28)	11 (6)	28	94	0.001
Friable	7 (24)	25 (13)	24	87	NS
Erythema	7 (24)	17 (9)	24	91	0.02
Ectropion	5 (17)	12 (6)	17	93	NS
Cervicitis—presence of any one of the following: mucopus, friable, ectropion, erythema	16 (55)	44 (23)	55	77	<0.001

NS = not significant.

A further classification of >10 PMNs and ≤10 PMNs was examined. There was a statistically significant difference when the results were examined by these criteria. The >10 PMN group correlated with *C trachomatis* infection and the ≤10 PMN group with absence of infection (p=0.02). However, the sensitivity and specificity was unacceptably low for both parameters. These results do not support the use of the PMN count alone for the presumptive diagnosis of *C trachomatis*.

#### CORRELATION BETWEEN *C TRACHOMATIS*, CLINICAL FINDINGS, AND THE NUMBER OF PMNs (TABLES 2, 3)

Mucopurulent endocervical discharge and endocervical erythema were statistically significant independent indicators for the presence of *C trachomatis* (p<0.001 and p=0.02 respectively). Friability and ectropion alone were not indicators of infection. Any of the following clinical findings were exhibited by 55% of infected subjects: mucopurulent endocervical discharge, friability, erythema, or ectropion, versus only 23% of those not infected (p<0.001).

Among the subjects with >10 PMN/HPF and mucopurulent discharge, 17% were observed to have *C trachomatis* (p=0.002). Significant observations were also seen between *C trachomatis* and the other clinical signs of cervicitis in the presence of >10 PMN/HPF. Although the specificities were acceptable the sensitivities were quite low. These findings indicate that the combination of clinical information and number of polymorphonuclear cells may be useful as a screening mechanism for *C trachomatis* infection.

#### ASSOCIATION OF *C TRACHOMATIS* INFECTION WITH PATIENT HISTORY

Although two thirds of the participants utilised some form of contraception, only 37% included barrier contraception. However, neither the use of contraception nor the use of barrier methods were predictors for the presence or absence of *C trachomatis*. Similarly, the phase of the menstrual cycle did not affect the identification of *C trachomatis*.

Table 3 Clinical findings and PMN/HPF counts in relation to *Chlamydia trachomatis* infection

Clinical findings and PMN/HPF counts	<i>C trachomatis</i>		Sensitivity (%)	Specificity (%)	p Value
	Pos n=29 (%)	Neg n=191 (%)			
>10 PMN/HPF + mucopus	5 (17)	4 (2)	17	98	0.002
>10 PMN/HPF + friable	4 (14)	7 (4)	14	96	0.04
>10 PMN/HPF + erythema	4 (14)	4 (2)	14	98	0.01
>10 PMN/HPF + ectropion	3 (10)	2 (1)	10	98	0.02

#### Discussion

The goal of this study was to clarify the usefulness of endocervical Gram smears in the diagnosis of *C trachomatis* and *N gonorrhoeae* infection. However, since only a single isolate of *N gonorrhoeae* was identified, no analysis was done for *N gonorrhoeae*. It is important for the clinician to be able to recognise signs of cervicitis to assist in early diagnosis and to help decrease *C trachomatis* associated morbidity such as salpingitis, infertility, ectopic pregnancy, pelvic inflammatory disease, neonatal pneumonia, and conjunctivitis.<sup>2-4 6 7 9 11 12</sup> The diagnosis of cervical chlamydial infection utilising the Gram smear and clinical findings demonstrated limited usefulness in this study.

The sensitivity and specificity of newly available laboratory analysis for diagnosis of *C trachomatis* has been continually changing. At the initiation of this study nucleic acid amplification was not available. It is currently accepted that this methodology is the most sensitive and specific. DFA was chosen to compare with culture owing to its improved sensitivity compared with enzyme immunoassays and the ability to determine specimen adequacy from the sample.

In relation to other reported studies, 55% of our slides were judged inadequate, compared with 16% and 24% reported by other authors.<sup>1 8</sup> Our finding was unexpected and definitely limits the diagnostic potential of screening endocervical Gram smears. It is suspected that the samples contained vaginal material because of improper collection methods. Forceps were not always available to clean the cervix, which may have resulted in an inappropriate sample. It is possible to contaminate the swab with vaginal secretions either upon entry or exit of the cervix. Whatever the cause, this finding reinforces the necessity of having the tools available for skilled, trained staff to perform such procedures to ensure quality specimens are collected for analysis.

Our ability to identify *C trachomatis* infection did not appear to be affected by menstrual cycle or method of contraception. These results are in agreement with those of Mosicki

*et al* who also did not note a significant difference in *C trachomatis* isolation with the phase of menses or the method of birth control.<sup>9</sup>

Various studies support the use of Gram stained smears as screening tools for identifying high risk women for *C trachomatis* infections. Brunham *et al* reported that  $\geq 10$  PMN/HPF was associated with the isolation of *C trachomatis* infection.<sup>1</sup> As reported by Katz, subjects with  $\geq 10$  PMN/HPF were twice as likely to have a positive *C trachomatis* culture, but an optimal cut-off for the number of PMNs was not found.<sup>8</sup> Moscicki *et al* concluded a cut-off of  $\geq 5$  PMN/HPF was effective in determining the presence of *C trachomatis*.<sup>9</sup> Knud-Hansen *et al* demonstrated limitations of the Gram stain and leucocyte esterase dipsticks to diagnose cervical infection.<sup>13</sup> They also concluded no suitable PMN cut-off was shown to be able to predict the presence of *C trachomatis* or *N gonorrhoeae*. We observed the presence of PMNs alone on endocervical smears was not indicative of *C trachomatis* infection. However, the Gram smear did permit specimen quality to be analysed. This is a recommended evaluation to ensure continued proper specimen collection for not only Gram stained smears, but also DFA and culture.

Gram smear information together with the clinical findings were also evaluated. We demonstrated statistically significant data when  $\geq 10$  PMN/HPF were found in the presence of mucopurulent endocervical discharge, friability, ectropion, and erythema. Unfortunately this same data did not result in high sensitivities although the specificity was acceptable.

Clinically significant signs of cervicitis vary between studies. Moscicki *et al* found a significant association between *C trachomatis* infection and the presence of erythema and oedema of ectopy, but not with mucopus and friability in a non-STD population.<sup>9</sup> Both Katz, and Brunham *et al* found mucopurulent discharge significant in relation to *C trachomatis* isolation.<sup>1,8</sup> Katz also notes ectopy to be an independent predictor of *C trachomatis* infection.<sup>1</sup> Theijls demonstrated that the absence of fragility/erythema could not be used to exclude *C trachomatis* infection in a youth outpatient clinic.<sup>6</sup> We witnessed a statistically significant association for the isolation of *C trachomatis* with the presence of mucopus and erythema when analysed individually. When the signs of cervicitis were grouped together we also documented significant associations, but again the sensitivity and specificity were unacceptable.

Overall, the results reported here do not support the use of endocervical Gram smears or clinical signs alone as definitive diagnostic tools for the presence of *C trachomatis* infection. However, they may be used as an aid to identify a population at high risk of *C trachomatis* and in need of early treatment regardless of diagnostic tests. The use of endocervical Gram smears may be especially useful in resource poor settings or in situations where advanced diagnostics are not available.

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*Contributors:* BR designed and supervised the study; MB assisted in the study design and specimen analysis; LM was the principal investigator, performed the specimen analysis, interpreted the data, and was the principal author of the paper.

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