Point-of-care management of sexual transmitted infections
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Citation for published version (APA):

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CHAPTER 3

Point-of-care management of urogenital Chlamydia trachomatis via Gram-stained smear analysis in male high-risk patients. Diagnostic accuracy and cost-effectiveness before and after changing the screening indication at the STI Clinic in Amsterdam

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Sex Transm Infect. 2015;91(7):479-84
ABSTRACT

Objectives To measure the effect of changing the POC testing algorithm of urogenital chlamydia for all male high-risk patients to those with only symptoms with respect to: diagnostic accuracy, loss to follow-up, correctly managed consultations and costs.

Methods Retrospective comparison of the diagnostic accuracy and cost-effectiveness of Gram stained urethral smear analysis for the POC management of urogenital Chlamydia trachomatis infections. Between 2008-2009 Gram stained urethral smear analysis was offered to all men irrespective of symptoms; between 2010-2011 only to those with symptoms. The Aptima CT assay was the reference diagnostic test.

Results The number of examined Gram stained smears in the two periods was respectively 7185 (2008-2009 period) and 18852 (2010-2011 period). The sensitivity of the Gram stain analysis was respectively 83.8% (95%CI 81.2-86.1) and 91.0% (95%CI 89.5-92.3) (p<0.001). The specificity was respectively 74.1% (95%CI 73.0-75.2) and 53.1% (95%CI 51.8-54.4) (p<0.001). The positive predictive value (PPV) was low in both periods, respectively 31.7% (95%CI 29.8 -33.6) and 35.6% (95%CI 34.1 -37.1) (p=0.002), whereas the negative predictive value (NPV) was high, respectively 97.0% (95%CI 96.4 -97.4) and 95.4% (95%CI 94.6 to 96.1) (p=0.002). The loss to follow-up rate between 2008-2009 and 2010-2011 was respectively 1.8% (95%CI 1.0 -2.9) versus 2.3 % (95%CI 1.7 -3.0) (p=0.36). There was a small difference in overtreatment, 68.0% (95%CI 66.0 -69.8) versus 64.1% (95%CI 62.6 -65.5) (p=0.001). The cost per correctly managed consultation was 14.3% lower in the 2010-2011 period (€ 94.31 versus € 80.82). The percentage of delayed treated infections was significantly lower in the 2008-2009 period (10.5%) compared with the 2010-2011 period (22.8%)(p<0.001).

Conclusions With a high sensitivity in male high-risk patients, the Gram stained urethral smear is a useful POC test to detect urogenital Chlamydia trachomatis. When offered only to men with urogenital symptoms the specificity decreases but the cost per correctly managed consultation is reduced with 14.3% without a significant difference in loss to follow-up but with a significantly higher rate of delayed treatment.
INTRODUCTION

*Chlamydia trachomatis* (CT) urogenital infection is a common sexually transmitted infection (STI) causing life threatening conditions like ectopic pregnancy and a high burden of morbidity like infertility and pelvic inflammatory disease.\(^1\) In 2005, according to WHO, there were approximately 98 million adults infected with CT and it was estimated that yearly 101 million new cases of CT occurred globally.\(^2\)

Point-of-care (POC) tests are important in the management of STIs because they allow the clinician to provide immediate test results and treatment.\(^3\) Recently, several companies have developed lipopolysaccharide based POC tests that provide rapid results for the detection of CT. However, low sensitivity (25–65%) precludes more widespread use in clinical settings.\(^4\) \(^5\)

In most STI clinics male patients are treated promptly for CT if a non-gonococcal urethritis (NGU) is diagnosed. NGU in men is characterised by discharge and urethral symptoms such as dysuria or urethral itching, but may be asymptomatic.\(^6\) NGU is based on the microscopic analysis of a smear from the urethra or urine sediment. NGU can be indicative for CT infections but other causative organisms such as *Mycoplasma genitalium* (MG), *Ureaplasma urolyticum*, *Trichomonas vaginalis*, anaerobic colon flora or herpes simplex virus may be involved. In many settings NGU is diagnosed if more than five polymorph nucleated leucocytes (PMNL) per high power field (hpf) are seen in a urethral Gram-stained smear, in the absence of intracellular negative diplococci. In the Netherlands >10 PMNLs/hpf is used as a threshold to diagnose NGU. For female high-risk patients a Gram-stained smear of genital samples is not a suitable POC test for urogenital CT because of the low positive predictive value.\(^7\)

Irrespective of symptomatology all high-risk male patients who visited the STI Outpatient Clinic of the Public Health Service of Amsterdam before February 2010 were offered a Gram-stained smear as a POC test for CT and *Neisseria gonorrhoeae* (NG). Since February 2010, only symptomatic high-risk patients were offered a Gram-stained smear, because of structural understaffing of the POC laboratory. Here we compared the Gram stain analysis in the POC management of urogenital CT in male high-risk patients in these two time periods. We evaluated the diagnostic accuracy, loss to follow up, percentage correctly managed consultations and the costs. The data set used in the current study was collected and described in an earlier study where we investigated the Gram stain analysis for POC management of urogenital NG.\(^8\)
METHODS

Study setting
The STI Outpatient Clinic in Amsterdam, the Netherlands, is a nurse-led clinic that offers free STI screening and treatment. Annually approximately 38,000 screenings are performed. During on-line enrolment patients are stratified into different risk groups. Patients are classified as high-risk if one of the following criteria is met: having STI-related symptoms, notified of an STI by a sexual partner, paid for sexual contact, men who had sex with men or uninsured patients from sub-Saharan Africa. More information about the study population can be found in the earlier published study about POC management of urogenital NG.

Study design and selection of patients
We performed a retrospective analysis of data from the electronic patient file. Since all data were collected for routine purposes and anonymised before the analysis, ethical clearance was not sought. Only male high-risk patients were selected. The male low-risk patients were excluded from the study because this group was not offered Gram stain examination. Some patients visited the STI clinic more than once and were offered a new standard testing procedure each time. The number of patients used in our calculations refers to the number of consultations and not to the number of unique patients.

Consultations with a missing or failing Gram stain and/or confirmation test result were excluded from the analysis. Consultations that took place on days that Gram stain examination was not available (because of laboratory understaffing) were also excluded. NGU diagnoses based on urine sediment were excluded from the diagnostic accuracy analysis. In the period between 1 January 2008 and 31 December 2009 (referred to as the 2008–2009 period) urethral Gram-stained smears for light microscopic examination were obtained from all male patients who were identified as high-risk irrespective of signs and/or symptoms. Between 12 February 2010 and 31 December 2011 (referred to as the 2010–2011 period), Gram stain analysis was performed only in male high-risk patients with urogenital signs or symptoms (discharge, painful and/or frequent urination).

Gram stain analysis and confirmation testing
If >10 PMNL/hpf were seen (in the absence of intracellular Gram-negative diplococci) in at least 3 different hpfs under a light microscope a presumptive diagnosis of NGU was made. In the case of a negative result, a first-void urine sample was examined under a light microscope after centrifugation at 1000
rpm for 3 min (urine sediment) and if more than 10 PMNL/hpf were seen in the urine also the presumptive diagnosis of NGU was made. Those diagnosed with NGU were given an instant oral dose of 1000 mg azithromycin plus post-test counselling and contact tracing. If Gram-negative diplococci were seen in the PMNLs, patients were presumptively treated for NG with ceftriaxone 500 mg intramuscularly and for CT with azithromycin 1000 mg orally. Patients with a Gram stain result of <10 PMNLs/hpf were not treated at the initial visit but were managed 1 week later when the definite results (serological, culture and nucleic acid amplification test) became available. Aptima CT assay (Genprobe, USA) was used as the standard reference test for urogenital CT. Samples for reference testing were obtained from a first void urine sample. More information about the management of high-risk patients is described in the online supplementary data and also in an earlier published study.²

**Confirmed and treated infections, prompt and delayed treatment, loss to follow-up and overtreatment**

The percentage of confirmed (by standard test) and treated infections was calculated as all confirmed CT infections treated at our clinic, out of all confirmed CT infections.

The percentage of promptly tested and correctly treated infections was calculated as all confirmed CT infections treated at the initial visit out of all confirmed CT infections. The proportion of delayed treated infections was calculated as all infections treated at the return visit out of all confirmed CT infections.

The percentage of loss to follow-up was calculated as the proportion of confirmed CT infections that were not treated at our clinic within 12 weeks after the definite diagnosis was available (upon three attempts to inform the patient), out of all confirmed CT infections. The percentage of overtreatment was calculated as those who received treatment upon a false positive Gram stain (negative Aptima CT assay) out of all infections that had to be treated upon a positive Gram stain.

**Costs per consultation and per correctly managed consultation**

Costs of the consultations were estimated from a health services perspective. Costs included were direct staff time (salary plus benefits), clinic space, supplies, overhead and medication. Costs for the patient, like loss of productivity due to waiting hours, were not accounted for. We calculated the cost per consultation by dividing the total costs by all consultations. To combine both the costs and the diagnostic accuracy in one outcome the cost per correctly managed consultation...
was calculated by dividing the total costs by the number of correctly managed consultations. Incorrect management of CT was defined as delayed treatment (treatment after the first visit), no treatment at all (loss to follow-up) or overtreatment (a positive Gram stain result without confirmed CT). The remaining consultations fell into the group of correctly managed consultations, that is, treated presumptively at the clinic upon a confirmed (by standard test) CT infection or those not treated with a negative standard test result. More information about the costs calculations can be found in the online supplementary data and table S1.

**Urethritis caused by NG**

Gram-stained smear analysis was also used for the detection of urogenital NG. In case a presumptive NG diagnosis was made, patients were treated with ceftriaxone plus azithromycin to cover NG and CT. During the 2010–2011 period, Gram-stained smear analysis was offered only to symptomatic patients. Since in men urogenital NG is more frequently symptomatic then urogenital CT, we calculated the prevalence of NG in both periods to exclude possible bias caused by the presumptive treatment of NG infections.

**Analysis**

Statistical analyses were done using Stata/SE V.12.1 for Windows and IBM SPSS Statistics V.21. Differences in proportions between the two study periods with 95% CIs were tested for the equality of proportions using large-sample statistics (binomial approximation) in the two study periods. Data of the consultation costs were analysed in Excel.

**RESULTS**

In the 2008–2009 period 30 079 consultations were performed in men, of which 20 492 (68.1%) were considered high-risk (figure 1). After exclusion, the final analysis set consisted of 7185 (23.9%) high-risk consultations.

In the 2010–2011 period 30 460 consultations were performed in men, of which 20 349 (66.8%) were considered high-risk. After exclusion the final analyses set consisted of 18 852 (61.9%) high-risk consultations.

Although the percentage of consultations included in the analysis is much higher in the 2010–2011 period as compared with the 2008–2009 period (mainly because of exclusion of the consultations in the 2008–2009 period because of days with lack of laboratory staff), the general characteristics like
sex, age, nationality and various risk factors in those included and excluded are comparable (see online supplementary table S2).

Figure 1. Flow chart of the management of urogenital Chlamydia trachomatis among high-risk male patients, STI outpatient clinic, Amsterdam, The Netherlands, 2008–2011

*Already treated for other reason (positive urine sediment, urogenital gonorrhoea, ano-rectal chlamydia/gonorrhoea, epididymitis, partner with chlamydia). †Four patients were not treated at the first visit (one patient was treated at the second visit, three patients were loss to follow-up). ‡Eight patients were not treated presumptively for unknown reason. §Four patients did read their results on internet, two patients didn’t. ¶Nine patients were not treated at the first visit (six patients were treated during the second visit and three patients were loss to follow-up). µFifteen patients were not treated presumptively at the first visit for unknown reason. ¥Five patients did read their results on internet, five patients didn’t. ψFourteen patients did read their results on internet, 14 patients didn’t. STI, sexually transmitted infection.

Diagnostic accuracy
The sensitivity of the Gram stain for CT was 83.8% (95% CI 81.2% to 86.1%) in the 2008–2009 period and 91.0% (95% CI 89.5% to 92.3%) in the 2010–2011 period (p<0.001).

The specificity of the Gram stain for CT was 74.1% (95% CI 73.0% to 75.2%) in the 2008–2009 period and 53.1% (95% CI 51.8% to 54.4%) in the 2010–2011 period (p<0.001).
The positive predictive value was low in both periods; 31.7% (95% CI 29.8% to 33.6%) in the 2008–2009 period and 35.6% (95% CI 34.1% to 37.1%) in the 2010–2011 period. In both periods there was a comparable high negative predictive value of 97.0% (95% CI 96.4% to 97.4%) and 95.4% (95% CI 94.6% to 96.1%), respectively. Detailed information about diagnostic accuracy can be found in the online supplementary table S3.

**Confirmed and treated infections, prompt and delayed treatment, loss to follow-up and overtreatment**

The proportion of confirmed CT infections treated at the clinic was comparably high in both periods: 98.2% vs 97.7% (p=0.26) (table 1). In the 2008–2009 period the percentage of promptly treated infections based on Gram stain POC management was significantly higher compared with the 2010–2011 period, respectively, 87.7% and 74.9% (p<0.001). Consequently the percentage of delayed treated infections was significantly lower in the 2008–2009 period (10.5%) compared with the 2010–2011 period (22.8%) (p<0.001).

There was no statistically significant difference in the loss to follow-up percentage between both periods: 1.8% (95% CI 1.0% to 2.9%) vs 2.3% (95% CI 1.7% to 3.0%) (p=0.36), but a small statistically significant difference in the percentage overtreated, 68.0% (95% CI 66.0% to 69.8%) vs 64.1% (95% CI 62.6% to 65.5%) (p=0.001) (table 1).


Table 1. Confirmed* and treated urogenital *Chlamydia Trachomatis* (Ct) infections, promptly treated and delayed treated infections, loss to follow-up and overtreated, in high-risk male patients, STI Outpatient Clinic, Amsterdam, the Netherlands, 2008-2011

<table>
<thead>
<tr>
<th></th>
<th>Confirmed Ct treated†</th>
<th>Promptly treated§</th>
<th>Delayed treated‡</th>
<th>Loss to follow-up¶</th>
<th>Over-treatment**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008-2009</td>
<td>885††/901 (98.2%)</td>
<td>790‡‡/901 (87.7%)</td>
<td>95‡§/901 (10.5%)</td>
<td>16¶¶/901 (1.8%)</td>
<td>1619***/2382 (68.0%)</td>
</tr>
<tr>
<td>2010-2011</td>
<td>2121†††/2171 (97.7%)</td>
<td>1625‡‡‡/2171 (74.9%)</td>
<td>496§§§/2171 (22.8%)</td>
<td>50¶¶¶/2171 (2.3%)</td>
<td>2630****/4106 (64.1%)</td>
</tr>
</tbody>
</table>

*Confirmed with Aptima Ct assay (Genprobe, USA).
†Proportion (%) at the clinic treated CT infections, out of all Aptima CT assay confirmed infections.
‡Proportion (%) promptly (at initial visit) treated CT infections, out of all Aptima CT assay confirmed infections.
§Proportion (%) delayed (at return visit) treated CT infections, out of all Aptima Ct assay confirmed infections.
¶Proportion (%) of untreated CT infections (not treated at the STI outpatient clinic) out of all Aptima CT assay confirmed infections, 12 weeks after the confirmed diagnosis became available.
**Proportion (%) overtreated patients was those who received treatment upon a false positive Gram stain (negative Aptima CT assay) out of all infections that had to be treated upon a positive Gram stain.
††The cases with a confirmed CT that were treated at the clinic were calculated as follows (see left flowchart and legend of figure 1): 751+39 (those already treated)+1 (not according to point-of-care (POC) protocol treated at the second visit)=885.
‡‡The cases with a confirmed CT that were treated promptly (at initial visit) were calculated as follows (see left flowchart and legend of figure 1): 751+39 (in the ‘already treated’ boxes)=790.
§§The cases with a confirmed CT with delayed treatment (at the second visit) were calculated as follows (see left flowchart and legend of figure 1): 94 (treated at second visit)+1 (not according to POC protocol treated at the second visit)=95.
¶¶The loss to follow-up cases were calculated as follows (see left flowchart and legend of figure 1): 13 (in the ‘loss to follow-up box’)+3 (exceptions in the ‘already treated box’)=16.
***The cases that were overtreated based on a false positive Gram stain (see left flowchart and legend of figure 1): 1619 (in the ‘overtreated box’).
†††The cases with a confirmed CT that were treated at the clinic were calculated as follows (see right flowchart and legend of figure 1): 1452+37+136 (those already treated)+96+394 (those treated at the second visit)+6 (not according to POC protocol treated at the second visit)=2121.
‡‡‡The cases with a confirmed CT that were treated promptly (at initial visit) were calculated as follows (see right flowchart and legend of figure 1): 1452+37+136 (in the ‘already treated boxes’)=1625.
§§§The cases with a confirmed CT with delayed treatment (at the second visit) were calculated as follows (see right flowchart and legend of figure 1): 96+394 (treated at second visit)+6 (not according to POC protocol treated at the second visit)=496.
¶¶¶The cases of loss to follow-up were calculated as follows (see right flowchart and legend of figure 1): 12+35 (in the ‘loss to follow-up boxes’)+3 (3 exceptions in the ‘already treated box’)=50.
****The cases that were overtreated based on a false positive Gram stain (see right flowchart and legend of figure 1): 2630 (in the ‘overtreated box’).
Costs per consultation and per correctly managed consultation

The average cost per consultation was estimated to be €71.60 in the 2008–2009 period versus €67.20 in the 2010–2011 period, a savings of €4.40 or 6.2% per consultation. On the basis of 75.9% correctly managed CT infections in the first period and 83.2% correctly managed CT infections in the second period, the cost per correctly managed consultation was €94.31 in the first period compared with €80.82 in the second period, a savings of €13.49 or 14.3% per correctly managed consultation (table 2).

Table 2. Cost per consultation and per correctly managed consultation of urogenital Chlamydia Trachomatis (CT), among high-risk male patients, STI Outpatient Clinic, Amsterdam, the Netherlands, 2008-2011

<table>
<thead>
<tr>
<th>Study period</th>
<th>2008-2009</th>
<th>2010-2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cost (TC)</td>
<td>€514479.17</td>
<td>€1266876.18</td>
</tr>
<tr>
<td>Total number of consultations (N)</td>
<td>7185</td>
<td>18852</td>
</tr>
<tr>
<td>Total number of correctly managed consultations (%) (NCM)</td>
<td>5455 (75.9%)</td>
<td>15676 (83.2%)</td>
</tr>
<tr>
<td>Mean cost per consultation (TC/N)</td>
<td>€71.60</td>
<td>€67.20</td>
</tr>
<tr>
<td>TC of incorrect management*</td>
<td>€30957.46 (n=1730)</td>
<td>€50578.89 (n=3176)</td>
</tr>
<tr>
<td>Due to delayed treatment</td>
<td>€75.27 (n=95)</td>
<td>€393.00 (n=496)</td>
</tr>
<tr>
<td>Due to loss to follow up; no treatment</td>
<td>€12.68 (n=16)</td>
<td>€39.62 (n=50)</td>
</tr>
<tr>
<td>Due to overtreatment</td>
<td>€30869.51 (n=1619)</td>
<td>€50146.27 (n=2630)</td>
</tr>
<tr>
<td>Mean cost per correctly managed consultation</td>
<td>€94.31</td>
<td>€80.82</td>
</tr>
</tbody>
</table>

*Incorrect management of CT is defined as delayed treatment (treatment after the first visit), no treatment at all (loss to follow up after a positive nucleic acid amplification test (NAAT)) or overtreatment (based on a positive Gram stain result without confirmed CT). The remaining consultations fall into the group of correct management, that is, treated presumptively at the clinic upon a confirmed (by standard test) CT infection or those not treated with a negative standard test result. More information about the costs calculations can be found in the online supplementary data.

Urethritis caused by NG

In the 2008–2009 period 10.7% (95% CI 8.6% to 13.2%) (81/775) patients with a true positive Gram stain for CT had a co-infection with NG compared with 13.8% (95% CI 12.0% to 15.6%) (201/1461) of the patients with a true positive Gram for CT in the 2010–2011 period (p=0.043).

In the 2008–2009 period 14.1% (95% CI 12.4% to 15.9%) (229/1627) of the patients with a false positive Gram stain for CT turned out to have an NG infection compared with 20.6% (95% CI 19.1% to 22.2%) (546/2645) of
the patients with a false positive Gram stain for CT in the 2010–2011 period (p<0.001).

**DISCUSSION**

POC management is a highly valued public health principle to prevent sequelae, loss to follow-up consultations and ongoing transmission.\(^3\) Faced with budget cuts we had to economise our POC laboratory around 2010. Whereas before 2010 all high-risk patients were offered a Gram stain smear testing to diagnose urogenital CT promptly, only those with urogenital symptoms received POC management from 2010 onwards. This measure resulted in a cost reduction of 14.3% per correctly managed consultation of urogenital CT.

In this study we found a relatively high sensitivity of Gram stain analysis for urogenital CT in both periods (83.8% in the 2008–2009 period and 91.0% in the 2010–2011 period) compared with the sensitivity reported by other studies, ranging from 23% to 71%.\(^{10,11}\) The relative high sensitivities in our study could be due to a selective study population that consisted of high-risk patients of which the majority were men who have sex with men (respectively 56.5% and 64.8% of the study populations) compared with more general populations in other studies. Also different analysis techniques and thresholds used for a prompt NGU diagnosis (5 PMNLs/hpf in other studies vs 10 PMNLs/hpf in the present study) make a comparison on outcomes difficult.\(^{12}\) According to national guidelines the outpatient clinic in Amsterdam uses more than 10 PMNLs/hpf as a threshold for NGU whereas most international guidelines advise to use more than 5 PMNLs/hpf as a threshold. The higher threshold in our study could have resulted in a higher specificity but lower sensitivity.

The higher sensitivity of the Gram stain to detect CT in the 2010–2011 period (91.0%) compared with the 2008–2009 period (83.8%) could be explained by fewer false negative outcomes when urethral smear analysis was performed solely in symptomatic men. Probably symptomatic CT infections are more likely to cause Gram stain positive smear results as opposed to asymptomatic CT infections.

On the other hand, the specificity was relatively low in both periods (74.1% and 53.1%). This can be partly explained by infection with NG which may have caused symptomatic urethritis in many patients. The lower specificity in the 2010–2011 period (53.1%) compared with the 2008–2009 period (74.1%) is remarkable. A possible explanation is that CT infections are mostly asymptomatic in contrast with infections with urogenital NG. That also explains the higher
percentage of NG infections found among the patients with a false positive
Gram stain for CT in the 2010–2011 period when the Grams stained smear was
only offered to symptomatic patients (20.6% vs 14.1%, p<0.001).

Moreover, other micro-organisms we did not test for routinely could have
caus ed positive smear results and so could be responsible for the high amount
of false positivity in both periods. NGU is reported to be caused by CT in
15–40% of cases, MG in 15–25%, T. vaginalis in 5–15% and less commonly,
herpes simplex virus and adenovirus in 2–4%. Ureaplasma urealyticum has been
associated in some but not all studies.\textsuperscript{7,13,14} However in 20–50% of NGU cases
the aetiology remains unknown.\textsuperscript{15} Although not excluded, most MG infections
are symptomatic; the organism is found in only 5–6% of asymptomatic men.\textsuperscript{16,17}
Likewise, urogenital infections caused by herpes simplex virus and adenovirus
are to cause symptoms more frequently than on average in bacterial related
NGU.\textsuperscript{5} These non-detected pathogens can further explain the low specificity
found in the study.

The cost per correctly managed consultation was 14.3% lower (a difference of
\euro 13.49 per consultation) in the 2010–2011 period compared with the 2008–
2009 period. This difference can be partially explained by the higher sensitivity
in the 2010–2011 period resulting in a higher percentage of correctly managed
consultations. Moreover there was less overtreatment in the 2010–2011 period.
Also the relative decrease in the amount of Gram stains analyses performed
in the 2010–2011 period contributed in the reduction of the cost per correctly
managed consultation. Yet the percentage of delayed treatment doubled. In a
future mathematical modelling study we want to quantify ongoing transmission
due to delayed treatment.

The strength of our study is the analysis of a large data set of comparable
study populations over both periods. The outcomes of this study are in line with
the outcomes of an earlier published study in which we compared the cost-
effectiveness of the Gram stain in detecting urogenital NG in both periods.\textsuperscript{8}
In that study we also reported a lower cost of urethral Gram stain analysis
when offered solely to symptomatic high-risk patients as opposed to all patients
irrespective of symptomatology.

A limitation of both our studies is that we focused only on CT and NG as pos-
sible causative agents of urethritis. We could not evaluate urethral infections by
other micro-organisms known to cause urethritis. It is still debateable if microor-
ganisms like MG should be routinely screened for, or only in case of symptoms.
More prospective studies are needed to give additional insight in the pathology
and treatment of MG and other causative micro-organisms of NGU.\textsuperscript{16,17}
To summarise, Gram stain smear analysis as a POC test for urogenital CT in symptomatic high-risk men only is more cost-effective compared with the analysis of all men, irrespective of symptoms. Screening symptomatic men only, saved 14.3% per correctly managed consultation, resulted in a higher sensitivity but a lower specificity, less overtreatment and a comparable loss to follow-up. Since there is no accurate and affordable pathogen-specific POC test for CT available, Gram stain smear analysis remains the preferred test for the prompt management of urogenital CT in high-risk men.

**Key messages**

- **Microscopic analysis of Gram-stained urethral smears is the most reliable point-of-care (POC) test to date for the presumptive management of urogenital chlamydia in men.**
- **The Gram-stained smear POC system is accurate for the presumptive management of urogenital chlamydia in high-risk men.**
- **When offered only to high-risk patients with urogenital symptoms, the cost per correctly managed consultation is reduced by 14.3% with less overtreatment and comparable loss to follow-up but a higher rate of delayed treatment.**

**Acknowledgements**

The authors wish to thank Dewi Usmany, Arjen Speksnijder, Caspar Signet and Ineke Linde of the Public Health Laboratory for providing data of diagnostic costs, Sander Hoogenkamp of the financial department of the PHS of Amsterdam for providing data of human resources costs and Ente Rood of the Royal Tropical Institute for helping setting up the design of the study.

**Contributors**

HJ CdV, MSvR and MS were the authors responsible for the design of the study. MSvR and MB collected and interpreted the data of the diagnostic outcomes. SA did the statistical analysis of the diagnostic outcomes. KV collected and analysed the data of the costs. MB drafted the paper. HJ CdV, MS and WRF supervised the overall study. All authors reviewed and approved the final article.

**Funding**

This study was financed by the Research and Development fund of the Public Health Service of Amsterdam, no 2382.
Competing interests
None.

Ethics approval
All data used in this study was collected as part of routine management and anonymised before the analysis. Therefore ethical approval was not considered necessary.

Provenance and peer review
Not commissioned; externally peer reviewed.
REFERENCES


SUPPLEMENTARY DATA

Table S1. Costing parameters in the point-of-care management of urogenital Chlamydia Trachomatis, STI outpatient clinic, Amsterdam, The Netherlands, 2008-2011

<table>
<thead>
<tr>
<th>Activity</th>
<th>Description</th>
<th>Cost (EUR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consultation</td>
<td>Initial patient visit; nurse takes patient history and conducts physical exam and NAAT swab</td>
<td>12.11</td>
</tr>
<tr>
<td>Reference test (Nucleic Acid Amplification Test (NAAT*))</td>
<td>NAAT test by on-site laboratory</td>
<td>48.53</td>
</tr>
<tr>
<td>Gram test</td>
<td>Gram swab and prep for analysis by nurse; reading by lab technician; doctor diagnosis</td>
<td>3.77</td>
</tr>
<tr>
<td>Treatment of patient with either positive Gram stain result or positive NAAT test</td>
<td>Azithromycin 1000mg prescribed by doctor and administered by nurse; contact tracing and counseling by nurse</td>
<td>19.07</td>
</tr>
<tr>
<td>Gram negative result</td>
<td>Nurse informs patient test is gram negative; end of visit</td>
<td>0.81</td>
</tr>
<tr>
<td>Patient follow up in case of a false Gram negative result</td>
<td>Nurse attempts to contact patient up to three times for treatment</td>
<td>2.42 per attempt</td>
</tr>
</tbody>
</table>

*Aptima Ct assay (Genprobe, USA)*
Table S2. Characteristics of high-risk visitors, STI outpatient clinic, Amsterdam, The Netherlands, 2008-2011

<table>
<thead>
<tr>
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<td>Excluded from analyses</td>
<td>Included in analyses</td>
<td>Excluded from analyses</td>
<td>Included in analyses</td>
<td>Excluded from analyses</td>
</tr>
<tr>
<td>Confirmed Ct by reference test</td>
<td>[n (%)]</td>
<td></td>
<td>[n (%)]</td>
<td></td>
<td>[n (%)]</td>
<td></td>
<td>[n (%)]</td>
</tr>
<tr>
<td>Yes</td>
<td>1385 (10.4)</td>
<td>901 (12.5)</td>
<td>58 (5.3)</td>
<td>2171 (11.5)</td>
<td>16681 (88.5)</td>
<td></td>
<td>1360 (90.8)</td>
</tr>
<tr>
<td>No</td>
<td>11858 (89.1)</td>
<td>6284 (87.5)</td>
<td>1497</td>
<td></td>
<td>0</td>
<td></td>
<td>18852</td>
</tr>
<tr>
<td>Missing</td>
<td>64 (0.5)</td>
<td></td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Age in years Median (SD)</td>
<td>35.0 (10.8)</td>
<td>34.0 (11.0)</td>
<td>35.0 (11.0)</td>
<td>34.0 (11.5)</td>
<td>35.0 (11.0)</td>
<td>34.0 (11.5)</td>
<td>35.0 (11.0)</td>
</tr>
<tr>
<td>Nationality [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch</td>
<td>9091 (68.3)</td>
<td>4831 (67.2)</td>
<td>1022 (68.3)</td>
<td>12965 (68.8)</td>
<td>1187 (6.3)</td>
<td></td>
<td>631 (3.3)</td>
</tr>
<tr>
<td>Surinamese</td>
<td>797 (6.0)</td>
<td>530 (7.4)</td>
<td>97 (6.5)</td>
<td>1187 (6.3)</td>
<td>631 (3.3)</td>
<td></td>
<td>403 (2.1)</td>
</tr>
<tr>
<td>South-American</td>
<td>420 (3.2)</td>
<td>250 (3.5)</td>
<td>41 (2.7)</td>
<td>631 (3.3)</td>
<td>483 (2.6)</td>
<td></td>
<td>403 (2.1)</td>
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<tr>
<td>Eastern European</td>
<td>355 (2.7)</td>
<td>154 (2.1)</td>
<td>32 (2.1)</td>
<td>483 (2.6)</td>
<td>403 (2.1)</td>
<td></td>
<td>358 (1.9)</td>
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<tr>
<td>Sub-Saharan African</td>
<td>371 (2.8)</td>
<td>195 (2.7)</td>
<td>36 (2.4)</td>
<td>358 (1.9)</td>
<td>403 (2.1)</td>
<td></td>
<td>358 (1.9)</td>
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<tr>
<td>North African</td>
<td>276 (2.1)</td>
<td>168 (2.3)</td>
<td>28 (1.9)</td>
<td>358 (1.9)</td>
<td>358 (1.9)</td>
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<td>358 (1.9)</td>
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<tr>
<td>Asian</td>
<td>280 (2.1)</td>
<td>152 (2.1)</td>
<td>32 (2.1)</td>
<td>358 (1.9)</td>
<td>358 (1.9)</td>
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<td>358 (1.9)</td>
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<tr>
<td>Antillean</td>
<td>179 (1.3)</td>
<td>113 (1.6)</td>
<td>24 (1.6)</td>
<td>358 (1.9)</td>
<td>358 (1.9)</td>
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</tr>
<tr>
<td>Turkish</td>
<td>169 (1.3)</td>
<td>100 (1.4)</td>
<td>24 (1.6)</td>
<td>358 (1.9)</td>
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<tr>
<td>Other</td>
<td>1369 (10.3)</td>
<td>692 (9.6)</td>
<td>161 (10.8)</td>
<td>358 (1.9)</td>
<td>358 (1.9)</td>
<td></td>
<td>358 (1.9)</td>
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<tr>
<td>Sex worker [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>145 (1.1)</td>
<td>68 (0.9)</td>
<td>7 (0.5)</td>
<td>140 (0.7)</td>
<td>18712 (99.3)</td>
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<td>140 (0.7)</td>
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<tr>
<td>No</td>
<td>13162 (98.9)</td>
<td>7117 (99.1)</td>
<td>1490 (99.5)</td>
<td>140 (0.7)</td>
<td>18712 (99.3)</td>
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<td>140 (0.7)</td>
</tr>
<tr>
<td>Has paid for sex in past 6 months [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>467 (3.5)</td>
<td>215 (3.0)</td>
<td>51 (3.4)</td>
<td>401 (2.1)</td>
<td>18451 (97.9)</td>
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<td>401 (2.1)</td>
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<tr>
<td>No</td>
<td>12840 (96.5)</td>
<td>6970 (97.0)</td>
<td>1446 (96.6)</td>
<td>401 (2.1)</td>
<td>18451 (97.9)</td>
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<td>401 (2.1)</td>
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Table S2. Characteristics of high-risk visitors, STI outpatient clinic, Amsterdam, The Netherlands, 2008-2011 (continued)

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<tr>
<td></td>
<td>13307*</td>
<td>7185</td>
<td>1497</td>
<td>18852</td>
</tr>
<tr>
<td>Alerted by partner [n (%)]:</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2474 (18.6)</td>
<td>1355 (18.9)</td>
<td>312 (20.8)</td>
<td>3980 (21.1)</td>
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<tr>
<td>No</td>
<td>10833 (81.4)</td>
<td>8530 (81.1)</td>
<td>1185 (79.2)</td>
<td>14872 (78.9)</td>
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<tr>
<td>Complaints [n (%)]:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7494 (56.3)</td>
<td>4332 (60.3)</td>
<td>1209 (80.8)</td>
<td>9237 (49.0)</td>
</tr>
<tr>
<td>No</td>
<td>5813 (43.7)</td>
<td>2853 (39.7)</td>
<td>288 (19.2)</td>
<td>9615 (51.0)</td>
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<tr>
<td>Man who had sex with men in the past 6 months [n (%)]:</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>7954 (59.7)</td>
<td>4060 (56.5)</td>
<td>742 (49.6)</td>
<td>12216 (64.8)</td>
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<tr>
<td>No</td>
<td>5353 (40.2)</td>
<td>3125 (43.5)</td>
<td>755 (50.4)</td>
<td>6636 (35.2)</td>
</tr>
</tbody>
</table>

* In the 2008-2009 period relatively more high-risk patients were excluded because of the exclusion of days when Gram stain examination was not available because of lack of laboratory staff.
Table S3. Positive and negative results of the Gram stain versus the standard reference test (Nucleic Acid Amplification Test [NAAT] *) for urogenital *Chlamydia Trachomatis* in men, STI outpatient clinic, Amsterdam, the Netherlands, 2008-2011

<table>
<thead>
<tr>
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<th>2010-2011</th>
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<tbody>
<tr>
<td></td>
<td>NAAT*</td>
<td>Total</td>
<td>NAAT</td>
<td>Total</td>
</tr>
<tr>
<td>Gram</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>755</td>
<td>1627</td>
<td>2382</td>
<td>1461</td>
</tr>
<tr>
<td></td>
<td>146</td>
<td>4657</td>
<td>4803</td>
<td>145</td>
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<tr>
<td></td>
<td>901</td>
<td>6284</td>
<td>7185</td>
<td>1606</td>
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<tr>
<td></td>
<td>2382</td>
<td>2645</td>
<td>4106</td>
<td>7302</td>
</tr>
<tr>
<td></td>
<td>1606</td>
<td>7302</td>
<td>8908</td>
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*Aptima Ct assay (Genprobe, USA)*
CHAPTER 3

Patient management

Gram stains from urethral smears in men were interpreted using high power field (hpf) light microscopic examination by an experienced laboratory technician on the POC laboratory. All permanent employed technicians (n=5) were trained at an intermediate to higher level of education for medical laboratory technology and had between 3 and 15 years of experience working in the POC laboratory of the STI clinic.

If more than 10 polymorph nucleated leucocytes (PMNL)/per high powerful field (hpf) were seen (in the absence of intracellular Gram negative diplococci) in at least three different fields under the light microscope a presumptive diagnosis of non-gonococcal urethritis (NGU) was made. In the case of a negative result, the cell pellet of a first-void urine sample (after centrifugation at 1000 rpm for 3 minutes) was examined under the light microscope and if more than 10 PMNL/hpf were seen in the urine, a presumptive diagnose of NGU was also made. Those diagnosed with NGU were given an oral dose of 1000 mg Azithromycin the same day, plus post-test counseling and contact tracing. If Gram negative diplococci were seen in the PMNL’s, patients were presumptively treated for both gonorrhoea (Ng) with Ceftriaxone 500 mg intramuscularly and urogenital chlamydia (Ct) with Azithromycin 1000 mg orally.

A presumptive treatment with Azithromycin was also given to partners of index patients with a documented Ct or NGU diagnosis, either in the electronic patient database or via an official notification slip. Also presumptive treatment for Ct was given to patients with a presumptive diagnose of epididymitis, non-gonoccal proctitis (NGP) or anorectal Ng infection.

The Aptima Ct assay (Genprobe, USA) was used as the gold standard reference test for urogenital Ct. Samples for reference testing were obtained from the first void urine sample.

The microbiological technicians who interpreted the NAAT were blind to the Gram stain results. All microbiological technicians (n=17) from the Public Health Laboratory finished a higher laboratory education and worked between 2 and 17 years with NAAT’s.

Within a week the definite diagnosis was based on the reference test result. In the case Ct was confirmed but the patient had not received Azithromycin or Doxycycline upon the initial visit he was summoned to the clinic for additional treatment and contact tracing. Those who did not show up were notified in 3 additional attempts via telephone, email, or surface mail within 12 weeks after the diagnosis was made. If all attempts failed, the patient was considered lost to follow up. Patients who had received Azithromycin or Doxycycline upon
the initial visit did not have to return to the clinic, unless other infections were diagnosed which required additional care.

**Costs per consultation and per correctly managed consultation**

Costs were estimated from a health services perspective. Time spent on procedures by nurses and doctors were based on expert opinion. Two different doctors and two different nurses who worked at least for one year at the STI clinic made an estimation of the activities related to the process of diagnosing and follow-up of Ct.

Time spent by laboratory technicians processing samples for Gram stain analysis was measured with a stopwatch. The financial department of the Public Health Service provided data of salaries, costs for materials, equipment and the overhead. All costs were reported in Euro (€), 2012 values, and exclusive of value added tax (VAT). Costs for the patient, like loss of productivity due to waiting hours, were not accounted for.

The cost of human resources was calculated by multiplying the time needed for each activity (in minutes) by the cost of one minute of working time of that staff type. Based on the above, the cost of the first visit was estimated to be 12.11 euro and the cost of treatment was estimated to be 19.07 euro. The cost of a Gram test was estimated to be 3.77 euro and the cost of a NAAT to be 48.53 euro. The costs spent on contacting patients (by phone or mail) who did not return for treatment was estimated to be 2.42 euro per attempt. The costs of each segment of a consultation were estimated and then multiplied by the actual number of patients receiving that part of the consultation in each of the two time periods, and then summed to give the total cost for each period. The total cost was divided by the number of consultations and number of correctly managed consultations to give the mean cost per consultation and the mean cost per correctly managed consultation. We calculated the cost per consultation and the cost per correctly managed consultation. Incorrect management of chlamydia is defined as delayed treatment (treatment after the first visit), no treatment at all (loss to follow-up after a positive NAAT), or over-treatment (based on a false positive Gram stain result). The remaining consultations fall into the group of correct management.