Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in an STI population: performances of the Presto CT-NG assay, the Lightmix Kit 480 HT CT/NG and the COBAS Amplicor with urine specimens and urethral/cervicovaginal samples

T A Schuurs,1 S P Verweij,2 J F L Weel,1 S Ouburg,2 S A Morré2,3

**ABSTRACT**

**Objectives:** This study assessed the performances of the Presto CT-NG assay, the Lightmix Kit 480 HT CT/NG and the COBAS Amplicor for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* detection.

**Design:** A cross-sectional study design.

**Setting:** Izore, Centre for Diagnosing Infectious Diseases in Friesland, the Netherlands, tested samples sent from regional sexually transmitted infection (STI) outpatient clinics and regional hospitals from the province Friesland, the Netherlands.

**Participants:** Samples were collected from 292 men and 835 women. These samples included 560 urine samples and 567 urethral/cervicovaginal samples.

**Primary and secondary outcome measures:** The primary outcome measure is *C. trachomatis* infection. No secondary outcome measures are available.

**Results:** The sensitivity, specificity, positive predicative value (PPV) and negative predicative value (NPV) for *C. trachomatis* detection in urine samples using the Presto CT-NG assay were 100%, 99.8%, 98.1% and 100%, respectively; for the Lightmix Kit 480 HT CT/NG: 94.2%, 99.8%, 96.1% and 99.4%, respectively; for the COBAS Amplicor: 92.3%, 99.6%, 96% and 99.2%, respectively. The sensitivity, specificity, PPV and NPV for *C. trachomatis* detection in urethral/cervicovaginal swabs using the Presto CT-NG assay and the COBAS Amplicor were 100%, 99.8%, 97.7% and 100%, respectively; for the Lightmix Kit 480 HT CT/NG: 100%, 99.6%, 97.7% and 100%, respectively. Calculations for *N. gonorrhoeae* could not be made due to a low prevalence.

**Conclusions:** All three assays had a high sensitivity, specificity, PPV and NPV for *C. trachomatis*, with best performance for the Presto CT-NG assay.

**INTRODUCTION**

Urogenital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the most prevalent bacterial sexually transmitted infections (STIs) in the Netherlands.1 In women, both infections are associated with severe sequelae including pelvic inflammatory disease, tubal scarring and tubal infertility.2,3 In Western society, highly sensitive and specific DNA or RNA amplification tests to detect *C. trachomatis* and *N. gonorrhoeae* are commercially available, and have increased detection rates as compared with conventional techniques including culture.4-6 A variety of clinical specimens, that is, urine specimens and cervicovaginal, anorectal or oropharyngeal swabs, can be used for STI detection and cost-saving test strategies have been described.2,7 Until recently, the COBAS Amplicor (Roche, California, USA) was the most widely used system for *C. trachomatis* and *N. gonorrhoeae* detection in the Netherlands. Newly developed dual detection systems for *C. trachomatis* and *N. gonorrhoeae* are implemented in Europe in the past 2 years including the Presto CT-NG assay (Goffin...
Molecular Technologies, Houten, the Netherlands) and the Lightmix Kit 480 HT CT/NG (TIB MOLBIOL, Berlin, Germany).

The aim of this prospective study was to compare the performances of the Presto CT-NG assay, the Lightmix Kit 480 HT CT/NG and the COBAS Amplicor in urine specimens and urethral/cervicovaginal samples for the detection of *Chlamydia trachomatis* and *N gonorrhoeae* in patients visiting general practitioners, gynaecologists and dermatovenerologists for symptoms most commonly generated by an STI.

**MATERIALS AND METHODS**

**Clinical specimens**

Urine specimens (n=560, 238 men and 322 women) and urethral/cervicovaginal swabs (n=567, 54 men and 513 women) were obtained from 292 men and 835 women. Urethral samples were obtained from men only. Samples were sent to Izore for routine STI testing by regional hospitals and general practitioners. Samples were obtained in the period from March to May 2010. An overview of the study design is given in figure 1.

**DNA isolation**

DNA was isolated with MP96 (Roche) according to the manufacturer’s protocol. DNA extraction from the urine samples and swabs for the COBAS Amplicor was performed on the COBAS platform.

**C trachomatis and N gonorrhoeae testing**

*C trachomatis* and *N gonorrhoeae* detection was performed with the Presto CT-NG assay (Goffin Molecular Technologies), the Lightmix Kit 480 HT CT/NG (TIB MOLBIOL) and the COBAS Amplicor (Roche). All tests were performed according to the protocols provided by the respective manufacturers. Owing to cross-reactivity with other *Neisseria* spp, the COBAS Amplicor-positive results were confirmed with *opla* PCR. Two qualified technicians performed the tests and were blinded for the results.

**Discrepancy analysis and statistical analysis**

Samples identified as *C trachomatis* positive or *C trachomatis* negative with the Presto CT-NG assay, the Lightmix Kit 480 HT CT/NG and the COBAS Amplicor were defined as true positives and true negatives, respectively, using an alloyed gold standard: If two of three tests were positive, the sample was considered positive. If only one test was positive, the sample was considered negative. The same algorithm was used for *N gonorrhoeae*.

To calculate the sensitivity, specificity, positive predictive value (PPV) and the negative predictive value (NPV), we used the alloyed gold standard as reference. The 95% Wilson Binomial CIs were calculated for the sensitivities, specificities, PPVs and NPVs.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity 95% CI</th>
<th>Specificity 95% CI</th>
<th>PPV 95% CI</th>
<th>NPV 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine <em>C trachomatis</em></td>
<td>100.0 0.9932 to 1.000</td>
<td>99.8 0.9886 to 0.9996</td>
<td>97.7 0.9611 to 0.9865</td>
<td>100.0 0.9932 to 1.000</td>
</tr>
<tr>
<td>Presto CT-NG assay</td>
<td>94.2 0.9195 to 0.9585</td>
<td>99.6 0.9404 to 0.9733</td>
<td>97.7 0.9611 to 0.9865</td>
<td>99.4 0.9834 to 0.9978</td>
</tr>
<tr>
<td>Lightmix Kit 480 HT CT/NG</td>
<td>99.8 0.9897 to 0.9996</td>
<td>99.8 0.9897 to 0.9996</td>
<td>97.7 0.9611 to 0.9865</td>
<td>100.0 0.9933 to 1.000</td>
</tr>
<tr>
<td>COBAS Amplicor</td>
<td>99.8 0.9897 to 0.9996</td>
<td>99.8 0.9897 to 0.9996</td>
<td>97.7 0.9611 to 0.9865</td>
<td>100.0 0.9933 to 1.000</td>
</tr>
<tr>
<td>Urethral/cervicovaginal <em>C trachomatis</em></td>
<td>100.0 0.9933 to 1.000</td>
<td>99.8 0.9933 to 1.000</td>
<td>97.7 0.9611 to 0.9865</td>
<td>100.0 0.9933 to 1.000</td>
</tr>
</tbody>
</table>

NPV, negative predictive value; PPV, positive predictive value.
RESULTS

The overall prevalence for *C. trachomatis* and *N. gonorrhoeae* in this study was 8.1–8.5% and 0.8–0.9%, respectively. Since the number of *N. gonorrhoeae*-positive samples was very limited, we could not reliably calculate sensitivity, specificity, PPV and NPV.

Using the Presto CT-NG assay, *C. trachomatis* DNA was detected in 53 of 560 urine specimens and in 43 of 567 urethral/cervicovaginal specimens, while the Lightmix Kit 480 HT CT/NG and the COBAS Amplicor resulted in 51 and 40, and 50 and 43 *C. trachomatis* positives, respectively. The sensitivity, specificity, PPV and NPV for *C. trachomatis* are summarised in Table 1.

For *N. gonorrhoeae*, the Presto CT-NG assay detected 3 of 560 urine specimens and 7 of 567 urethral/cervicovaginal specimens. The Lightmix Kit 480 HT CT/NG and the COBAS Amplicor (followed by opaA confirmation PCR on *N. gonorrhoeae* positives) detected 3 and 7, and 1 and 8 of urine specimens and urethral/cervicovaginal specimens, respectively.

DISCUSSION

In the Netherlands, the prevalence of STI is stable or slightly increasing. Besides education, accurate diagnostics are also essential for prevention of further spreading of STI in the healthy population. Therefore, diagnostic tests, detecting STIs, should display maximum sensitivity whereas false-positives have to be precluded at any time.

We compared the performance of the Presto CT-NG assay (Goffin Molecular Technologies), the Lightmix Kit 480 HT CT/NG (TIB MOLBIOL) and the COBAS Amplicor (Roche) to an alloyed gold standard, defined as a positive result in at least two of three tests. The used samples were urine and urogenital swabs. The results show high specificity, sensitivity, PPV and NPV for all tests, with the Presto CT-NG assay as best overall performance for *C. trachomatis*.

Owing to the low prevalence of *N. gonorrhoeae*, we were not able to calculate specificity, sensitivity, PPV and NPV. The Presto CT-NG assay and the Lightmix Kit 480 HT CT/NG detected *N. gonorrhoeae* equally, but for a definitive validation more samples are needed. The overall prevalence of *N. gonorrhoeae* in this study population was 0.8–0.9%, which is in concordance with a recent report of the National Institute for Public Health and the Environment stating that Friesland province has an *N. gonorrhoeae* prevalence of 1–2%. The prevalence of *C. trachomatis* in this study population is slightly lower than the reported annual prevalence: 8.1–8.5% vs 12–14%. This observed difference may be explained by the fact that the National Institute for Public Health and the Environment includes data from all STI outpatient clinics in the Netherlands, whereas this study uses samples from a single region.

Performance of the COBAS Amplicor regarding *C. trachomatis* detection in this study was comparable with its performance in other studies. In these other studies, similar high sensitivities, specificities, PPVs and NPVs were achieved, as we found in this study.

To conclude, we find high specificity, sensitivity, PPV and NPV for all tests for *C. trachomatis*, with the Presto CT-NG assay having the best overall performance.

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Contributors TAS was involved in study design, collected and analysed the data. SPV analysed the data and was involved in writing the manuscript. JFLW designed the study, collected the data and critically reviewed the manuscript. SO supervised the data analyses, supervised writing and critically reading of the manuscript. SAM designed the study, was involved in overall supervision and critically reading the manuscript.

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Competing interests SAM, employed by the VU University Medical Center has been involved in the technical development of the Presto CT-NG assay (Goffin Molecular Technologies, Houten, the Netherlands) via Microbiome Ltd, a spin-in company of the VU University Medical Center, Amsterdam, the Netherlands.

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Data sharing statement No additional data are available.

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