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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

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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 predictive 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.

Strengths and limitations of this study

- All CT-NG tests were run at the same time in the same setting reducing potential operator bias.
- Although our sample size was quite large, our study had a limited number of Neisseria gonorrhoeae-positive samples, so no sensitivities, specificities, positive predictive values and negative predicting values were calculated.
- Three CT/NG tests were used on all available samples using both urine and urethral/cervicovaginal samples.
- A slight bias may have been introduced by making use of an alloyed gold standard.
- The New CE-IVD marked Presto CT-NG performs equally well as the FDA approved Roche 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

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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 *C trachomatis* and *N gonorrhoeae* in patients visiting general practitioners, gynaecologists and dermatovenereologists 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 *opa* 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.[8] The 95% Wilson Binomial CIs were calculated for the sensitivities, specificities, PPVs and NPVs.[9]

Table 1 Sensitivity, specificity, PPV and NPV for the three assays for *Chlamydia trachomatis* detection

	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Urine *C trachomatis*								
Presto CT-NG assay	100.0	0.9932 to 1.000	99.8	0.9896 to 0.9996	98.1	0.9660 to 0.9895	100.0	0.9932 to 1.000
Lightmix Kit 480 HT CT/NG	94.2	0.9195 to 0.9585	99.8	0.9896 to 0.9996	96.1	0.9416 to 0.9741	99.4	0.9834 to 0.9978
COBAS Amplicor	92.3	0.8980 to 0.9423	99.6	0.9864 to 0.9988	96.0	0.9404 to 0.9733	99.2	0.9806 to 0.9967
Urethral/cervicovaginal *C trachomatis*								
Presto CT-NG assay	100.0	0.9933 to 1.000	99.8	0.9897 to 0.9996	97.7	0.9611 to 0.9865	100.0	0.9933 to 1.000
Lightmix Kit 480 HT CT/NG	100.0	0.9933 to 1.000	99.6	0.9865 to 0.9988	97.7	0.9611 to 0.9865	100.0	0.9933 to 1.000
COBAS Amplicor	100.0	0.9933 to 1.000	99.8	0.9897 to 0.9996	97.7	0.9611 to 0.9865	100.0	0.9933 to 1.000

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

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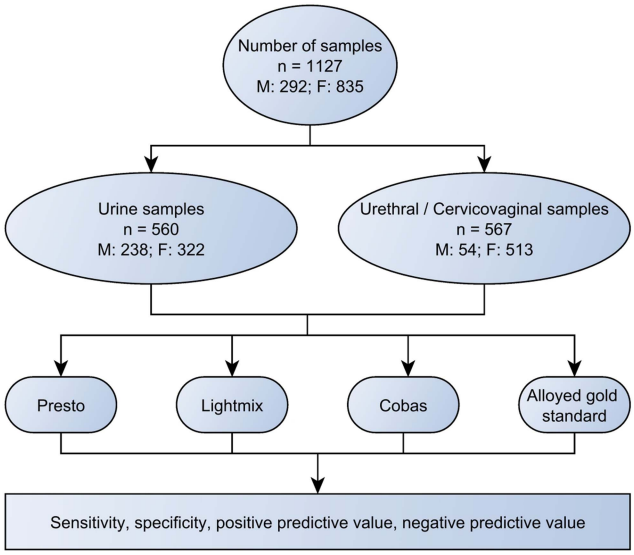

Figure 1 Study flow diagram. The diagram shows the included samples divided by gender and sample type. All samples were tested with three CT/NG assays and an "alloyed gold standard" was generated from these results. Sensitivity, specificity, positive predicative value, and negative predictive value were calculated for all tests.

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.[1] 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%.[1] The prevalence of *C trachomatis* in this study population is slightly lower than the reported annual prevalence: 8.1–8.5% vs 12–14%.[1] 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.[10 11] 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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