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

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

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Article summary

Article focus

- To evaluate performances of three commercially available nucleic acid amplification tests (NAAT) in the detection of *Chlamydia trachomatis* and *Neisseria genorrhoeae*: Presto, Lightmix Kit 480 HT CT/NG, and COBAS Amplicor.

Key messages

- Prevalence of *C. trachomatis* and *N. gonorrhoeae* is stable or slightly increasing in the Netherlands. Well functioning diagnostic tools are essential within the healthcare.
- All three NAATs evaluated in this study are highly sensitive and specific for detection of *C. trachomatis*, with best performance for Presto.

Strengths and limitations

- Although our sample size was quite large, our study had a limited number of *N. gonorrhoeae* positive samples, so no sensitivities, specificities, positive predictive values and negative predicting values were calculated for *N. gonorrhoeae*.

Abstract

Objectives

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

Design

It is a prospective study design.

Setting

Izore, Centre for Diagnosing Infectious Diseases in Friesland, the Netherlands, tested samples sent from regional STI outpatient clinics and regional hospital 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

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 Presto were 100,0%, 99,8%, 98,1%, 100,0%, respectively; for Lightmix Kit 480 HT CT/NG: 94,2%, 99,8%, 96,1%, 99,4%, respectively; for COBAS Amplicor: 92,3%, 99,6%, 96,0%, 99,2%, respectively. The sensitivity, specificity, PPV, and NPV for *C. trachomatis* detection in urethral/cervicovaginal swabs using Presto and COBAS Amplicor were 100,0%, 99,8%, 97,7%, 100,0%, respectively; for Lightmix Kit 480 HT CT/NG: 100,0%, 99,6%, 97,7%, 100,0%, 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 (STI) in the Netherlands [1]. 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 to conventional techniques including culture [4-6]. A variety of clinical specimens, *i.e.* 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, COBAS Amplicor (Roche, CA, 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 last two 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 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 dermato-venereologists for complaints most commonly generated by an STI.

Material 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. Samples were sent to Izore, Centre for Diagnosing Infectious Diseases in Friesland, the Netherlands, for routine STI testing by regional hospitals and general practitioners. Samples were obtained in the period from March - May 2010.

DNA isolation

DNA was isolated with MP96 (Roche) according to 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, Houten, The Netherlands), the Lightmix Kit 480 HT CT/NG (TIB MOLBIOL, Berlin, Germany), and COBAS Amplicor (Roche, CA, USA). All tests were performed according to the protocols provided by the respective manufacturers. Due to cross-reactivity with other *Neisseria spp*., 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 Presto, TIB MOLBIOL, and COBAS Amplicor were defined as true positives and true negatives, respectively using an alloyed gold standard: If two out 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]. 95% Wilson Binomial Confidence Intervals were calculated for the sensitivities, specificities, PPVs, and NPVs [9].

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 assay *C. trachomatis* DNA was detected in 53 out of 560 urine specimens and in 43 out of 567 urethral/cervicovaginal specimens, while the Lightmix Kit 480 HT CT/NG and 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 summarized in Table 1.

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

Table 1. Sensitivity, specificity, PPV and NPV for the three assays.

Urine *C. trachomatis*								
	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Presto CT/NG assay	100,0	0.9932 – 1.000	99,8	0.9896 – 0.9996	98,1	0.9660 – 0.9895	100,0	0.9932 – 1.000
Lightmix Kit 480 HT CT/NG	94,2	0,9195 - 0,9585	99,8	0,9896 - 0,9996	96,1	0,9416 - 0,9741	99,4	0,9834 - 0,9978
Cobas Amplicor	92,3	0,8980 - 0,9423	99,6	0,9864 - 0,9988	96,0	0,9404 - 0,9733	99,2	0,9806 - 0,9967

Urethral/cervicovaginal *C. trachomatis*								
	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Presto CT/NG assay	100,0	0,9933 - 1,000	99,8	0,9897 - 0,9996	97,7	0,9611 - 0,9865	100,0	0,9933 - 1,000
Lightmix Kit 480 HT CT/NG	100,0	0,9933 - 1,000	99,6	0,9865 - 0,9988	97,7	0,9611 - 0,9865	100,0	0,9933 - 1,000
Cobas Amplicor	100,0	0,9933 - 1,000	99,8	0,9897 - 0,9996	97,7	0,9611 - 0,9865	100,0	0,9933 - 1,000

Discussion

In the Netherlands, the prevalence of STI is stable or slightly increasing [1]. Besides education also accurate diagnostics is essential for prevention of further spreading of STI in the healthy population. Therefore diagnostic tests, detecting STI's, 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 out 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.

Due to the low prevalence of *N. gonorrhoeae*, we were not able to calculate specificity, sensitivity, PPV, and NPV. 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 to its performance in other studies [10;11]. In these other studies, similar high sensitivities, specificities, PPVs, and NPVs were achieved, as we do in this study. Due to too small number specificity, sensitivity, PPV and NPV could not be calculated for *N. gonorrhoeae*.

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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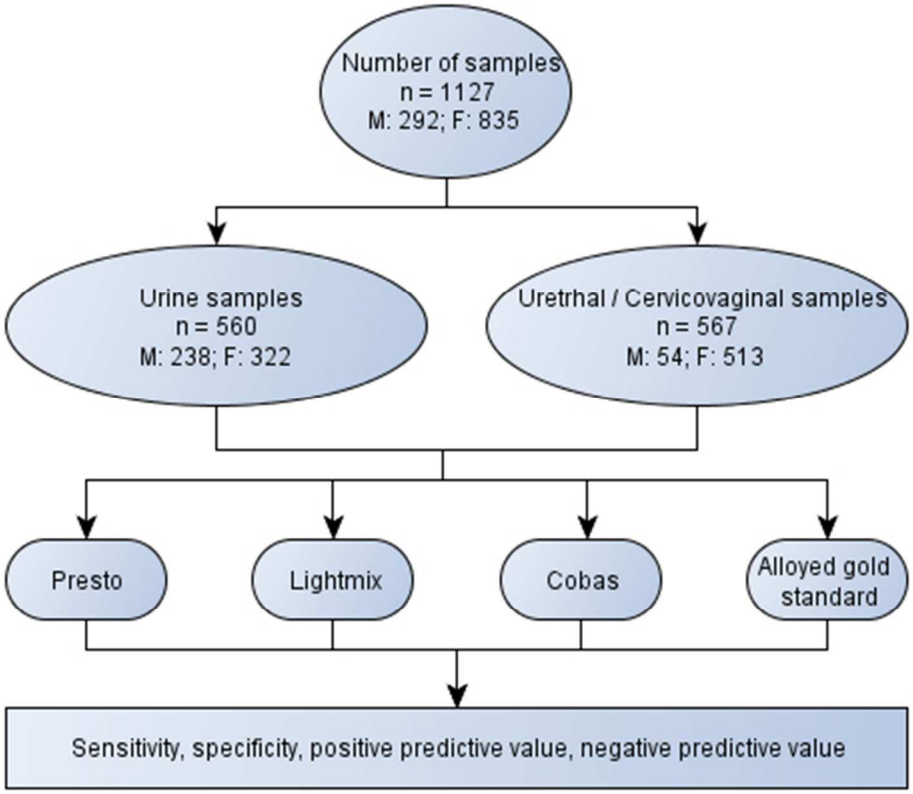
STARD checklist for reporting of studies of diagnostic accuracy

(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	4
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	5
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	5
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	5
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	4
Test methods	7	The reference standard and its rationale.	6
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	5, 6
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	5, 6
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	n/a
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	n/a
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	6
	13	Methods for calculating test reproducibility, if done.	n/a
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	n/a
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	7
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	7
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	n/a
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	n/a
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	n/a
	20	Any adverse events from performing the index tests or the reference standard.	n/a
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	8
	22	How indeterminate results, missing data and outliers of the index tests were handled.	n/a
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	n/a
	24	Estimates of test reproducibility, if done.	n/a
DISCUSSION	25	Discuss the clinical applicability of the study findings.	9



Flow chart of the study
169x148mm (72 x 72 DPI)

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

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Tables : 1

figures: 1

Abstract

Objectives

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

Design

It is a cross-sectional study design.

Setting

Izore, Centre for Diagnosing Infectious Diseases in Friesland, the Netherlands, tested samples sent from regional 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

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 Presto were 100,0%, 99,8%, 98,1%, 100,0%, respectively; for Lightmix Kit 480 HT CT/NG: 94,2%, 99,8%, 96,1%, 99,4%, respectively; for COBAS Amplicor: 92,3%, 99,6%, 96,0%, 99,2%, respectively. The sensitivity, specificity, PPV, and NPV for *C. trachomatis* detection in urethral/cervicovaginal swabs using Presto and COBAS Amplicor were 100,0%, 99,8%, 97,7%, 100,0%, respectively; for Lightmix Kit 480 HT CT/NG: 100,0%, 99,6%, 97,7%, 100,0%, 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.

Article summary

Article focus

- To evaluate performances of three commercially available nucleic acid amplification tests (NAAT) in the detection of *Chlamydia trachomatis* and *Neisseria genorrhoeae*: Presto CT-NG assay, Lightmix Kit 480 HT CT/NG, and COBAS Amplicor.

Key messages

- Prevalence of *C. trachomatis* and *N. gonorrhoeae* is stable or slightly increasing in the Netherlands. Well functioning diagnostic tools are essential within the healthcare.
- All three NAATs evaluated in this study are highly sensitive and specific for detection of *C. trachomatis*, with best performance for the Presto CT-NG assay.

Strengths and limitations

- Although our sample size was quite large, our study had a limited number of *N. gonorrhoeae* positive samples, so no sensitivities, specificities, positive predictive values and negative predicting values were calculated.

Introduction

Urogenital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the most prevalent bacterial sexually transmitted infections (STI) 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 to conventional techniques including culture [4-6]. A variety of clinical specimens, *i.e.* 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, COBAS Amplicor (Roche, CA, 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 last two 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 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 dermato-venereologists for complaints most commonly generated by an STI.

Material 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, Centre for Diagnosing Infectious Diseases in Friesland, the Netherlands, for routine STI testing by regional hospitals and general practitioners. Samples were obtained in the period from March - May 2010.

DNA isolation

DNA was isolated with MP96 (Roche) according to 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, Houten, The Netherlands), the Lightmix Kit 480 HT CT/NG (TIB MOLBIOL, Berlin, Germany), and COBAS Amplicor (Roche, CA, USA). All tests were performed according to the protocols provided by the respective manufacturers. Due to cross-reactivity with other *Neisseria spp*., 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 Presto CT-NG assay, Lightmix Kit 480 HT CT/NG, and COBAS Amplicor were defined as true positives and true negatives, respectively using an alloyed gold standard: If two out 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]. 95% Wilson Binomial Confidence Intervals were calculated for the sensitivities, specificities, PPVs, and NPVs [9].

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 out of 560 urine specimens and in 43 out of 567 urethral/cervicovaginal specimens, while the Lightmix Kit 480 HT CT/NG and 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 summarized in Table 1.

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

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

Urine *C. trachomatis*								
	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Presto CT-NG assay	100,0	0.9932 – 1.000	99,8	0.9896 – 0.9996	98,1	0.9660 – 0.9895	100,0	0.9932 – 1.000
Lightmix Kit 480 HT CT/NG	94,2	0,9195 - 0,9585	99,8	0,9896 - 0,9996	96,1	0,9416 - 0,9741	99,4	0,9834 - 0,9978
Cobas Amplicor	92,3	0,8980 - 0,9423	99,6	0,9864 - 0,9988	96,0	0,9404 - 0,9733	99,2	0,9806 - 0,9967

Urethral/cervicovaginal *C. trachomatis*								
	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Presto CT-NG assay	100,0	0,9933 - 1,000	99,8	0,9897 - 0,9996	97,7	0,9611 - 0,9865	100,0	0,9933 - 1,000
Lightmix Kit 480 HT CT/NG	100,0	0,9933 - 1,000	99,6	0,9865 - 0,9988	97,7	0,9611 - 0,9865	100,0	0,9933 - 1,000
Cobas Amplicor	100,0	0,9933 - 1,000	99,8	0,9897 - 0,9996	97,7	0,9611 - 0,9865	100,0	0,9933 - 1,000

Discussion

In the Netherlands, the prevalence of STI is stable or slightly increasing [1]. Besides education also accurate diagnostics is essential for prevention of further spreading of STI in the healthy population. Therefore diagnostic tests, detecting STI's, 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 out 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*.

Due to the low prevalence of *N. gonorrhoeae*, we were not able to calculate specificity, sensitivity, PPV, and NPV. Presto CT-NG assay and 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 to 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.

Acknowledgements

We would like to acknowledge Renske Minnema and Griet Verbeek (Izore, Centre for Infectious Diseases Friesland, Leeuwarden, The Netherlands) for the technical support. Prof.dr. S.A. Morré, 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.

Contributorship Statement

TAS: Study design, data collection, performed analyses

SPV Performed analyses, wrote manuscript

JFW study design, data collection, critically reviewing the manuscript

SO supervised data analyses, supervised writing, critically reading the manuscript

SAM study design, overall supervision, critically reading the manuscript.

Competing interests

None

Data sharing statement

No further data are available.

Reference List

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

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Abstract no. of words:	218 (max. 300)
Body text no. of words:	977 (max. 1000)
References:	11
Tables/figures:	2

Article summary

Article focus

- To evaluate performances of three commercially available nucleic acid amplification tests (NAAT) in the detection of *Chlamydia trachomatis* and *Neisseria genorrhoeae*: Presto CT-NG assay, Lightmix Kit 480 HT CT/NG, and COBAS Amplicor.

Key messages

- Prevalence of *C. trachomatis* and *N. gonorrhoeae* is stable or slightly increasing in the Netherlands. Well functioning diagnostic tools are essential within the healthcare.
- All three NAATs evaluated in this study are highly sensitive and specific for detection of *C. trachomatis*, with best performance for the Presto CT-NG assay.

Strengths and limitations

- Although our sample size was quite large, our study had a limited number of *N. gonorrhoeae* positive samples, so no sensitivities, specificities, positive predictive values and negative predicting values were calculated.

Abstract

Objectives

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

Design

It is a ~~prospective~~ cross-sectional study design.

Setting

Izore, Centre for Diagnosing Infectious Diseases in Friesland, the Netherlands, tested samples sent from regional 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

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 Presto were 100,0%, 99,8%, 98,1%, 100,0%, respectively; for Lightmix Kit 480 HT CT/NG: 94,2%, 99,8%, 96,1%, 99,4%, respectively; for COBAS Amplicor: 92,3%, 99,6%, 96,0%, 99,2%, respectively. The sensitivity, specificity, PPV, and NPV for *C. trachomatis* detection in urethral/cervicovaginal swabs using Presto and COBAS Amplicor were 100,0%, 99,8%, 97,7%, 100,0%, respectively; for Lightmix Kit 480 HT CT/NG: 100,0%, 99,6%, 97,7%, 100,0%, 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 (STI) 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 to conventional techniques including culture [4-6]. A variety of clinical specimens, *i.e.* 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, COBAS Amplicor (Roche, CA, 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 last two 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 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 dermato-venereologists for complaints most commonly generated by an STI.

Material 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, Centre for Diagnosing Infectious Diseases in Friesland, the Netherlands, for routine STI testing by regional hospitals and general practitioners. Samples were obtained in the period from March - May 2010.

DNA isolation

DNA was isolated with MP96 (Roche) according to 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, Houten, The Netherlands), the Lightmix Kit 480 HT CT/NG (TIB MOLBIOL, Berlin, Germany), and COBAS Amplicor (Roche, CA, USA). All tests were performed according to the protocols provided by the respective manufacturers. Due to cross-reactivity with other *Neisseria spp.*, 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 Presto CT-NG assay, Lightmix Kit 480 HT CT/NG~~TIB MOLBIOL~~, and COBAS Amplicor were defined as true positives and true negatives, respectively using an alloyed gold standard: If two out 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]. 95% Wilson Binomial Confidence Intervals were calculated for the sensitivities, specificities, PPVs, and NPVs [9].

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 out of 560 urine specimens and in 43 out of 567 urethral/cervicovaginal specimens, while the Lightmix Kit 480 HT CT/NG and 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 summarized in Table 1.

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

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

Urine *C. trachomatis*								
	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Presto CT/NG assay	100,0	0.9932 – 1.000	99,8	0.9896 – 0.9996	98,1	0.9660 – 0.9895	100,0	0.9932 – 1.000
Lightmix Kit 480 HT CT/NG	94,2	0,9195 - 0,9585	99,8	0,9896 - 0,9996	96,1	0,9416 - 0,9741	99,4	0,9834 - 0,9978
Cobas Amplicor	92,3	0,8980 - 0,9423	99,6	0,9864 - 0,9988	96,0	0,9404 - 0,9733	99,2	0,9806 - 0,9967

Urethral/cervicovaginal *C. trachomatis*								
	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Presto CT/NG assay	100,0	0,9933 - 1,000	99,8	0,9897 - 0,9996	97,7	0,9611 - 0,9865	100,0	0,9933 - 1,000
Lightmix Kit 480 HT CT/NG	100,0	0,9933 - 1,000	99,6	0,9865 - 0,9988	97,7	0,9611 - 0,9865	100,0	0,9933 - 1,000
Cobas Amplicor	100,0	0,9933 - 1,000	99,8	0,9897 - 0,9996	97,7	0,9611 - 0,9865	100,0	0,9933 - 1,000

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Discussion

In the Netherlands, the prevalence of STI is stable or slightly increasing [1]. Besides education also accurate diagnostics is essential for prevention of further spreading of STI in the healthy population. Therefore diagnostic tests, detecting STI's, 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 out 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*.

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Due to the low prevalence of *N. gonorrhoeae*, we were not able to calculate specificity, sensitivity, PPV, and NPV. Presto CT-NG assay and 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 to its performance in other studies [10;11]. In these other studies, similar high sensitivities, specificities, PPVs, and NPVs were achieved, as we founddo in this study. ~~Due to too small number specificity, sensitivity, PPV and NPV could not be calculated for *N. gonorrhoeae*.~~

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.

Acknowledgements

We would like to acknowledge Renske Minnema and Griet Verbeek (Izore, Centre for Infectious Diseases Friesland, Leeuwarden, The Netherlands) for the technical support. Prof.dr. S.A. Morré, employed by the VU University medical center has been involved in the technical development of the Presto CT-NG assay ~~test~~ (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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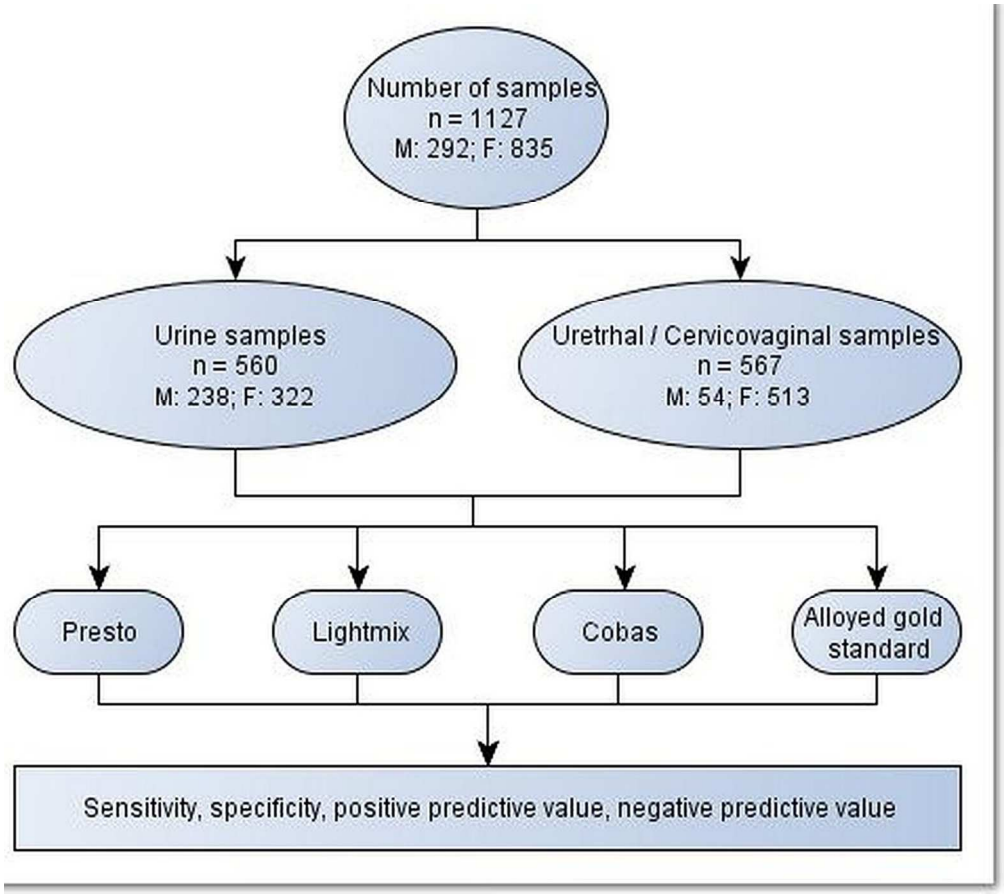
STARD checklist for reporting of studies of diagnostic accuracy

(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	5
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	65
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	65
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	65
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	54
Test methods	7	The reference standard and its rationale.	76
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	5, 66, 7
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	5, 6, 7
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	65
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	65
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	76
	13	Methods for calculating test reproducibility, if done.	n/a
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	n/a
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	65, 87
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	87
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	n/a
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	n/a
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	n/a
	20	Any adverse events from performing the index tests or the reference standard.	n/a
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	98
	22	How indeterminate results, missing data and outliers of the index tests were handled.	n/a
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	n/a
	24	Estimates of test reproducibility, if done.	n/a
DISCUSSION	25	Discuss the clinical applicability of the study findings.	109



Flow chart of the study
101x90mm (300 x 300 DPI)