



Comparison of GMT presto assay and Roche cobas® 4800 CT/NG assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in dry swabs



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ABSTRACT

Urogenital *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are the most prevalent bacterial STIs worldwide. Molecular tests are the standard for the detection of CT and NG, as these are difficult to culture. The recently introduced CE-IVD marked GMT Presto assay promises to be a valuable addition in CT and NG diagnostics. The advantage of the Presto assay is that it works on many PCR systems and the DNA can be isolated by any system. We compared the Presto assay to the widely used Roche cobas® 4800 CT/NG test for the detection of CT and NG in 612 vaginal and rectal dry collected swabs. Discrepant samples were tested by the TIB MOLBIOL Lightmix Kit 480 HT CT/NG assay. The alloyed gold standard was defined as two concurring Presto and cobas® 4800 results, or, with discrepant Presto and cobas® results, two concurring results of either test together with the Lightmix Kit 480 HT CT/NG assay. For the Presto assay, we observed 77 CT positive (13%) and 22 NG positive (3,6%) vaginal samples, and 41 CT positive (6,7%) and 11 NG positive (1,8%) rectal samples. For the cobas® 4800 assay, we observed 77 CT positive (13%) and 21 NG positive (3,4%) vaginal samples, and 39 CT positive (6,4%) and 11 NG positive (1,8%) rectal samples. Ten CT samples were discrepant between Presto and cobas® 4800 CT/NG assays, while two NG samples were discrepant. CT sensitivity in both assays was 100% compared to the alloyed gold standard. The sensitivity was 100% for both vaginal and rectal dry swabs, underlining the suitability of these sample types for detection of CT and NG. The Presto assay is therefore valuable for molecular detection of CT and NG in dry vaginal and rectal swabs.

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1. Introduction

Urogenital *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (CT) are the most prevalent bacterial sexually transmitted infections (STI) worldwide (Trienekens et al., 2012). CT has an increasing prevalence with almost 100 million new infections each year (Morré et al., 2009). The prevalence of NG is also increasing with an annual incidence of almost 31 million cases. CT and NG are both highly curable, which is why screening remains important to prevent late complications, such as ectopic pregnancy and pelvic inflammatory disease (PID), with considerable costs for society and psychological burden for women.

Highly sensitive and specific DNA or RNA amplification tests to detect CT and NG are commercially available, and have increased detection rates as compared to conventional techniques including culture. NAATs are described to be independent of bacterial viability and therefore

detect less false-negative results (Goessens et al., 1997; Quint et al., 2007; Hjelmevoll et al., 2008). A variety of clinical specimens, i.e. urine specimens and cervicovaginal, anorectal, or oropharyngeal swabs, can be used for STI detection (Morré et al., 1999, 2000). The Roche cobas® Amplicor system is a commonly used system for CT and NG detection in The Netherlands. The cobas® Amplicor is based on the detection of CT plasmid DNA; this system has recently been replaced by the cobas® 4800 assay which detects two CT gene targets: one in the cryptic plasmid and the other on the CT genome. Newly developed dual detection systems for CT and NG are implemented in Europe in the last decade including the Presto CT/NG assay which can be used on a variety of open PCR systems. In another study, we have recently (Schuurs et al., 2013) compared this new assay to the cobas® Amplicor using a cohort of 1128 clinical samples (561 urine samples and 567 urethral/cervicovaginal swabs) and obtained comparable sensitivities and specificities for CT. The NG incidence was too low to assess sensitivity and specificity. In the current study we compared the new Presto Assay with the currently widely used cobas® 4800 CT/NG test for the detection

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of CT and NG using dry collected vaginal and rectal swabs of South African women. Performances (sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)) were assessed for both assays and discrepancy analyses were performed with a third assay.

2. Methods

2.1. Sample selection

A cross-sectional study was conducted at primary healthcare facilities across the Mopani District, South Africa. 612 women, aged 18–49 years who reported to have been sexually active during the last 6 months were eligible and physician collected vaginal and rectal swabs were obtained (Copan Diagnostics, Brescia, Italy). Swabs were frozen for storage without buffer (dry swabs). Patient information was provided and written consent obtained (Peters et al., 2014).

A previous study described a prevalence of 16% genital Chlamydia and 10% for genital gonorrhoeae. Rectal prevalence was 7.1% for Chlamydia and 2.5% for gonorrhoeae (Peters et al., 2014).

2.2. Sample assessment

The samples were collected in Mopani District, South Africa and processed in Amsterdam, The Netherlands. Material from dry swabs was placed into 1 ml of sterile phosphate-buffered saline (PBS) and the tubes were vortexed. Then, 200 µl of each sample was used for DNA extraction and the rest was stored at -20°C for storage. DNA was extracted for each assay according to manufacturer's instructions.

For the Presto Assay, DNA was isolated by the High Pure Template Preparation Kit (Roche). *C. trachomatis* and *N. gonorrhoeae* were detected by the CE-IVD certified Presto test (Goffin Molecular Diagnostics, Houten, The Netherlands) according to the manufacturer's instructions, detection on the LightCycler II (Roche).

For the cobas® 4800 assay, DNA was isolated on the Roche X480 and the dual CT/NG detection on the Roche Z480.

2.3. Discrepancy analysis

Discrepant samples between Presto and cobas® 4800 were analyzed by the TIB MOLBIOL Lightmix Kit 480 HT CT/NG assay. The alloyed gold standard (Spiegelman et al., 1997) was defined as two concurring results for the Presto and cobas® 4800 tests, or, with discrepant results

between the Presto and cobas® assays, two concurring results of either test together with the Lightmix Kit 480 HT CT/NG assay.

3. Results

3.1. *C. trachomatis* detection

For the vaginal samples, 76 positive samples and 535 negative samples were concordant. Only two samples were discrepant. For the rectal samples, 35 positive samples and 567 negative samples were concordant. Ten samples were discrepant. See Fig. 1 for details.

3.2. *N. gonorrhoeae* detection

For the vaginal samples, 20 positive samples and 589 negative samples were concordant. Three samples were discrepant. For the rectal samples, 10 positive samples and 600 negative samples were concordant. 2 samples were discrepant. See Fig. 2 for details.

Table 1 shows the calculated sensitivity, specificity, positive predictive value, and negative predictive value for both assays and both anatomical sites calculated against the alloyed gold standard. Sensitivity, specificity, and positive and negative predictive value between the cobas® 4800 CT/NG Test and the Presto assay were comparable.

3.3. Detection of double infections

The Presto assay detected four concurring vaginal CT and NG infections, while the cobas® 4800 assay detected six concurring vaginal CT and NG infections. The overlap between the Presto and cobas® 4800 assays was four samples. In the anal samples the Presto assay detected six double infections, while the cobas® 4800 detected seven double infections. The overlap was six samples between both assays.

4. Discussion

In this study we compared the new Presto assay to the frequently used cobas® 4800 CT/NG test. The Presto assay showed comparable performance compared to the cobas® 4800 CT/NG Test regarding sensitivity specificity, positive and negative predictive values (see Table 1). This was the case for both vaginal and rectal dry swabs further underlining the suitability of these sample types for detection of infection with CT and NG.

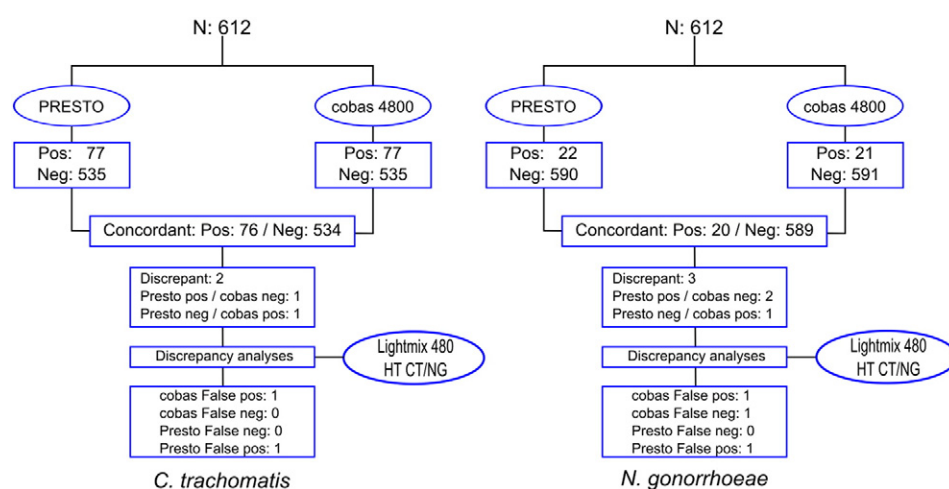


Fig. 1. Flow diagram of the results of vaginal CT and NG infections. The 612 samples, tested by the PRESTO and cobas® 4800 resulted in concordant and discrepant results. The Lightmix 480 HT CT/NG assay was used for discrepant samples and the gold standard was defined as two concurring results between the Presto and cobas® 4800 assays, or when these were discrepant, a concurring result between either Presto or cobas® 4800 assay and the Lightmix 480 HT CT/NG assay.

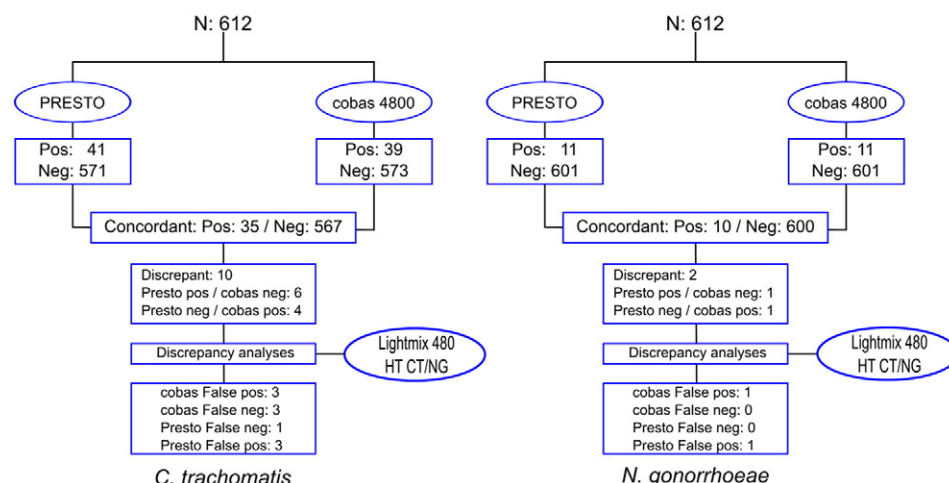


Fig. 2. Flow diagram of the results of anal CT and NG infections. The 612 samples, tested by the PRESTO and cobas® 4800 resulted in concordant and discrepant results. The Lightmix 480 HT CT/NG assay was used for discrepant samples and the gold standard was defined as two concurring results between the Presto and cobas® 4800 assays, or when these were discrepant, a concurring result between either Presto or cobas® 4800 assay and the Lightmix 480 HT CT/NG assay.

Accurate diagnostics are essential for prevention of further spreading of STI in the healthy population. Therefore diagnostic tests should display maximum sensitivity whereas false-positives have to be precluded at any time.

We have recently published the first study comparing the Presto to the Lightmix Kit 480 HT CT/NG and to the cobas® Amplicor and comparable results were obtained for CT (Schuurs et al., 2013). The sensitivity for *C. trachomatis* detection in urine samples using Presto versus Lightmix Kit 480 CT/NG versus cobas® Amplicor were 92.3–100.0%, respectively. The specificity for *C. trachomatis* detection in urine samples using Presto versus Lightmix Kit 480 CT/NG versus cobas® Amplicor were 99.6–99.8%, respectively. The PPV for *C. trachomatis* detection in urine samples using Presto versus Lightmix Kit 480 CT/NG versus cobas® Amplicor were 96.0–98.1%, respectively. The NPV for *C. trachomatis* detection in urine samples using Presto versus Lightmix Kit 480 CT/NG versus cobas® Amplicor were 99.2–100.0%, respectively.

The cobas® 4800 assay is a commonly used assay for detection. The cobas® 4800 assay was comparable to its performance in another study, with a sensitivity and specificity of 99% and 95%, respectively (Spiegelman et al., 1997; Jalal et al., 2007). The Abbott RealTime CT/NG Assay was also compared to the cobas® Amplicor CT/NG Assay (Cheng et al., 2011). This study found that the two assays are comparable for detection of *C. trachomatis* and *N. gonorrhoeae*. A study by Dize et al. showed that the Cepheid GeneXpert CT/NG assay and the Abbott m2000 RealTime CT assay perform as well as the single Roche Amplicor CT Assay for detection of *C. trachomatis*. The GeneXpert CT/NG assay

showed for both sensitivity and specificity 100% (Dize et al., 2013). The APTIMA Combo 2 assay showed comparable sensitivities and specificities with the results of this current and other assays (Gaydos et al., 2003). Fig. 3 shows a summary of several studies which examined comparison between assays.

We observed five samples with concurrent rectal CT and NG infections and six samples with concurrent vaginal CT and NG infections. The Cp values of the CT and NG tests in these samples were comparable to the Cp values in women with only a single CT or NG infection. In the rectal samples we observed one false negative CT test for the cobas® Amplicor CT/NG assay. However the sample numbers were too low for statistical conclusions on this difference.

In our study we used dry swabs. A study by Gaydos et al. describes the evaluation of dry and wet transported intravaginal swabs in detection of *C. trachomatis* and *N. gonorrhoeae*. This study concludes that the dry swab was as accurate as the wet swab (Gaydos et al., 2002). Another study by Eperon et al. concluded that swabs can be successfully transported in a dry state at ambient temperature without greatly altering specimen integrity (Eperon et al., 2013). Due to the prevalence we found, we can conclude that the dry collected vaginal swabs worked well for *C. trachomatis* and *N. gonorrhoeae*.

In conclusion, the new CE IVD certified Presto assay performance was comparable to the cobas® 4800 CT/NG Assay.

The weakness of this study was that number of samples tested could have been higher, but this was balanced by the high prevalence of infections. The strength of the study was the use of two sample sites using dry swabs providing new diagnostic insights.

Table 1

Sensitivity, specificity, positive predictive value, and negative predictive value for CT and NG for cobas® 4800 versus Presto.

		SENS%	95% CI	SPEC%	95% CI	PPV%	95% CI	NPV%	95% CI
Vaginal CT	Roche	100.0	99.4–100.0	99.8	99.0–100.0	98.7	97.5–99.3	100.0	99.4–100.0
	Presto	100.0	99.4–100.0	99.8	99.0–100.0	98.7	97.5–99.3	100.0	99.4–100.0
Vaginal NG	Roche	95.2 [#]	93.3–96.7	99.8	99.1–100.0	95.2	93.2–96.7	99.8	99.1–100.0
	Presto	100.0 [#]	99.4–100.0	99.8	99.1–100.0	95.5	93.5–96.8	100.0	99.4–100.0
Rectal CT	Roche	92.3 [*]	89.9–94.2	99.5	98.5–99.6	92.3	89.9–94.2	99.5	98.5–99.8
	Presto	97.4 [*]	95.9–98.4	99.5	98.5–99.9	92.7	90.3–94.5	99.8	99.1–100.0
Rectal NG	Roche	100.0	99.4–100.0	99.8	99.1–100.0	90.9	88.4–92.9	100.0	99.4–100.0
	Presto	100.0	99.4–100.0	99.8	99.1–100.0	90.1	88.4–92.9	100.0	99.4–100.0

SENS, sensitivity; CI, confidence interval; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value.

The alloyed gold standard was a concurring result between the Presto and cobas® 4800 assays, or when these were discrepant, a concurring result between either Presto or cobas® 4800 assay and the Lightmix 480 HT CT/NG assay. Sensitivity, specificity, PPV, and NPV for both assays and both anatomical sites were calculated against the alloyed gold standard.

[#] 1 sample difference in sensitivity analyses.

^{*} 2 sample difference in sensitivity analyses.

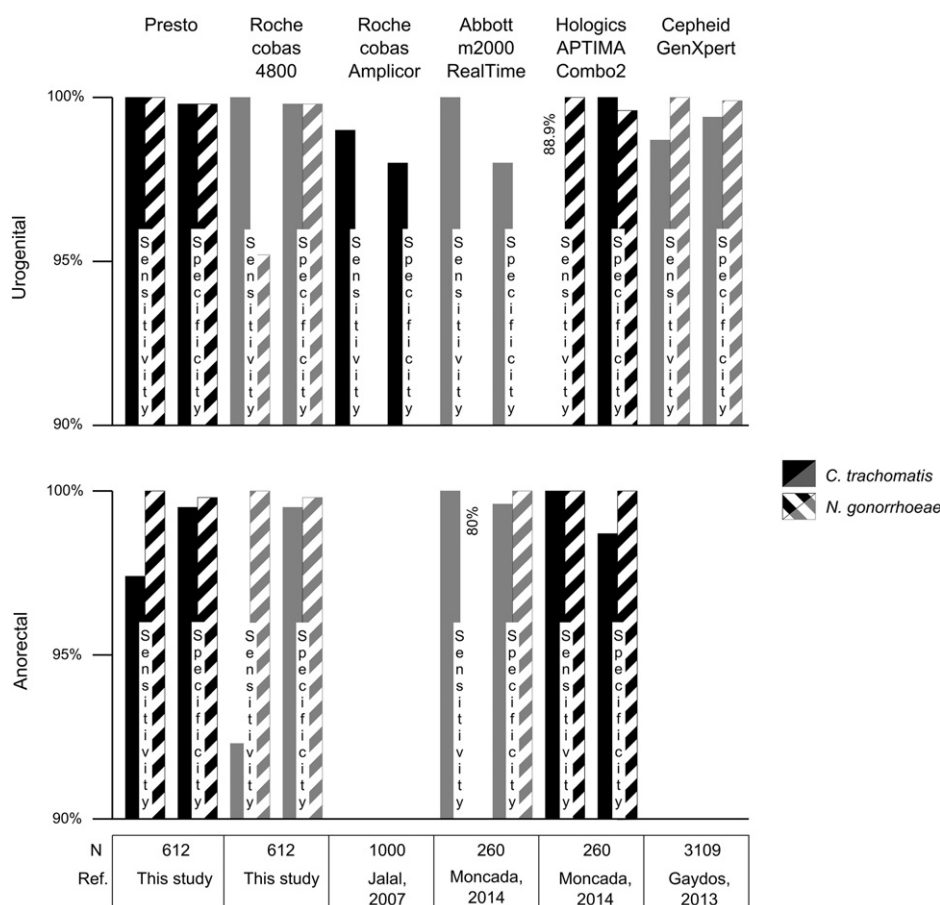


Fig. 3. Bar graph of sensitivities and specificities for the Presto CT/NG assay, the Roche cobas® 4800 CT/NG assay, the Roche cobas® Amplicor CT assay (Jalal et al., 2007), the Abbott m2000 RealTime CT/NG assay (Moncada et al., 2014), the Hologics APTIMA Combo2 CT/NG assay (Moncada et al., 2014), and the Cepheid GenXpert CT assay (Gaydos et al., 2013). Per assay two sets of bars are given: the first set represents *C. trachomatis* (solid bar) and *N. gonorrhoeae* (striped bar) sensitivity of the assay, and the second set represents *C. trachomatis* (solid bar) and *N. gonorrhoeae* (striped bar) specificity of the assay. Black and gray are used for visual distinction between bars of the assays. Top graph: Urogenital samples (swabs or urine); bottom graph: anorectal swabs. For the Roche cobas® Amplicor CT assay and the Cepheid GenXpert CT/NG assay no anorectal sensitivities and specificities were described. The Presto CT/NG assay, the Roche cobas® 4800 CT/NG assays were tested on 612 women compared to an alloyed gold standard (this study); the Roche cobas® Amplicor CT assay was performed on 1000 genital swab from men and women compared to an alloyed gold standard using an in-house PCR (Jalal et al., 2007); the Abbott m2000 RealTime CT/NG and Hologics APTIMA Combo2 CT/NG assays were performed on rectal swabs and urine of 260 MSM compared to an alloyed gold standard (APTIMA CT or NG) (Moncada et al., 2014); and the Cepheid GenXpert CT assay was performed on urogenital swabs and urine samples of 3109 men and women compared to an alloyed gold standard (APTIMA Combo 2 and ProbeTec ET CT/NG) (Gaydos et al., 2013).

In conclusion, this study showed comparable performances between new Presto assay to the frequently used cobas® 4800 CT/NG test. Both tests are suitable for detecting concurrent CT and NG infections at one infection site.

Competing interests

SAM, employed by the VU University Medical Center has been involved in the technical development of the Presto CT-NG assay (marketed by 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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