

BMJ Open Performance evaluation of the PelvoCheck CT/NG test kit for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

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ABSTRACT

Objective: Assessment of the performance of the PelvoCheck CT/NG test (Greiner-Bio-One GmbH) to detect *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) in first-void urine (FVU) of females.

Design: A cross-sectional study to compare the PelvoCheck CT/NG with COBAS TaqMan CT Test V.2.0 (Roche) for the detection of CT and with an in-house *porA*-based PCR for the detection of NG in FVU specimens. In addition, pools of 5 FVU specimens containing only CT-negative or 1 CT-positive and 4 CT-negative samples were tested. Abbott RealTime CT/NG was used as an additional test to resolve discordant results.

Setting: Samples sent from six laboratories were tested at the University Medical Center Hamburg.

Participants: Urine samples were from 1622 female patients attending gynaecological practices for chlamydia screening, another 120 urine samples were from patients pretested for NG at Synlab, Medical Service Center, Weiden GmbH. In addition, 50 urine samples spiked with various concentrations of reference material were used.

Results: For the detection of CT and NG, the sensitivity and specificity of the PelvoCheck CT/NG test were 98.8% and 100%, and 98.3% and 98.2%, respectively. The data obtained with the PelvoCheck CT/NG for pooled urine specimens resulted in a positive agreement of 90.9% and a negative agreement of 100%.

Conclusions: The PelvoCheck CT/NG assay is a suitable test method for the detection of CT and NG in female FVU samples, with sensitivity and specificity comparable with other Food and Drug Administration approved CT/NG nucleic acid amplification tests. To the best of our knowledge, this is the first commercial test system validated for the analysis of pooled urine specimens. No false-positive or invalid result was observed in 55 analysed pools. Nevertheless, 5 samples were false negative due to a target concentration below the limit of detection of the PelvoCheck CT/NG test as a consequence of pooling-associated dilution.

Strengths and limitations of this study

- The PelvoCheck *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) test and comparative assays were simultaneously performed on identical sets of samples in the same setting to minimise operator bias.
- Clinical specimens used in the study were derived from patients visiting gynaecological practices who were either participating in the national Chlamydia screening program or because of clinical symptoms, representing everyday conditions of sample collection.
- The PelvoCheck CT/NG test results were highly concordant with those of comparative assays; and the test performs equally as well as the Food and Drug Administration approved assays COBAS TaqMan CT Test V.2.0 (Roche) and Abbott RealTime CT/NG.
- Owing to the low CT prevalence, some CT-negative urine samples were spiked with different amounts of *C trachomatis* strain DSM-19131 serovar E (German collection of microorganisms). Although using various concentrations that occur in reality, spiking does not exactly reflect natural conditions of infection and may have introduced some bias.

INTRODUCTION

Worldwide *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are the most frequent sexually transmitted bacterial infections, with an estimated incidence of each numbering 108 million new infections annually.¹ In 2011, the European Centre for Disease Prevention and Control reported an incidence of 175/100 000 for CT and 12.6/100 000 for NG, based on reported cases from 25 to 28 European Union countries, respectively.² Accurate incidence data for Germany are lacking, as there is no general obligation to report CT and NG infections. Only in the federal state of Saxony are laboratories committed to report these

infections to local authorities. In 2011, 95 CT infections per 100 000 inhabitants and 13.7/100 000 NG infections were registered.³ These numbers, however, are likely to be an underestimation of true infection rates, as Saxony may not be representative of the whole of Germany. Beyond that, infections are not always symptomatic and thus may escape diagnosis.

In Germany, NG infections are detected predominantly in risk groups such as men having sex with men and in commercial sex workers.⁴ In contrast, CT infections affect the whole sexually active population and are detected most frequently in young individuals, aged <25 years.^{2 5} In women, most CT and NG infections are asymptomatic or cause subclinical disease, however, symptomatic and asymptomatic infections may both persist and induce severe sequelae including pelvic inflammatory disease, ectopic pregnancy and infertility.^{6 7} To reduce the burden of disease, many countries have established Chlamydia screening program that aim to identify infections and initiate early treatment.

In Germany, opportunistic screening for chlamydia was implemented in 2008. A test to detect a CT infection is offered to women up to 25 years of age, once a year. It must be performed in first-void urine (FVU) specimens using a nucleic acid amplification test (NAAT). With respect to cost savings, laboratories may combine up to five samples in one test. If this pool of samples is negative, all individual samples can be considered to be negative. If the pool is positive, all five samples have to be re-tested individually to identify the positive(s). However, the applicability of this pooling strategy is discussed controversially, as it may reduce sensitivity⁸ and it is incompatible with quality standards of microbiological diagnostics. Consequently, several guidelines do not recommend testing of pooled specimens.^{9–11}

Among all methods for direct detection, NAATs are generally considered as the method of choice to detect CT, due to the highest analytical and clinical sensitivity, and a specificity similar to culture.^{9 11 12} NAATs are also the most sensitive tests for NG, however, specificity may be reduced due to cross-reactivity with non-pathogenic *Neisseria*. This is of relevance when testing samples from anal or pharyngeal sites, which are frequently colonised with commensal *Neisseria*.^{13–16} Therefore, NG-positive NAAT results of anal or pharyngeal specimens should be confirmed by another NAAT or by culture.^{9 17} In addition, increasing rates of NG isolates with antimicrobial resistance, including third generation cephalosporins, have motivated inclusion of antimicrobial susceptibility testing of cultured isolates in the diagnostics of gonorrhoea.^{9 17} As a convenient approach, clinical material is collected from patients with symptoms suspicious of gonorrhoea for NAAT and/or culture just before starting empirical treatment. Asymptomatic patients may be first tested by NAAT and only in case of a positive result, bacterial culture should be performed with a second sample before starting treatment.¹⁷

A number of commercial NAATs for CT and NG are available. Many of them are designed as duplex assays analysing both pathogens.¹⁸ The recently developed PelvoCheck CT/NG test kit from Greiner-Bio-One GmbH also simultaneously analyses CT and NG and can be used for both urine and swabs. To evaluate the performance of the PelvoCheck CT/NG test in FVU, we examined urine samples obtained during Chlamydia screening by both PelvoCheck CT/NG and Roche COBAS TaqMan CT Test V.2.0 as a comparative NAAT, as well as FVU specimens pretested for NG in another laboratory by PelvoCheck CT/NG and an in-house PCR test based on *porA* gene sequences.

MATERIAL AND METHODS

Clinical specimens for testing NG

NG testing was performed with 120 FVU samples (60 positive and 60 negative) pretested at Synlab Medical Service Center GmbH, Weiden, Germany, using an in-house PCR assay targeting the gonococcal *opa* gene. The samples were collected from females during routine examinations. After testing, the remaining material was distributed into three parts used for the following PCR tests: (1) For PelvoCheck CT/NG, 3 mL of urine was transferred into specific collection and transport tubes (PelvoCheck Collection kit SAFE) and stored at 0–8°C. (2) For the *porA* based in-house PCR assay, at least 1 mL of the native urine sample was stored at –20°C. (3) For the Abbott RealTime CT/NG test, which was used to resolve discordant results of the former two tests, 3 mL urine were transferred to an Abbott multi-Collect Specimen Collection kit and stored at –20°C.

Clinical specimens for testing CT

CT testing was performed with 1622 FVU samples collected from female patients in Southern Bavaria attending gynaecological practices for chlamydia screening. Samples were accepted when the following inclusion criteria were fulfilled: women were aged 18–25 years, not menstruating at the time of urine collection, urine sample must be the first portion of 15–30 mL and the last micturition must be more than 1 h prior. In addition, 50 samples spiked with various concentrations of positive reference material (CT strain DSM-19131 serovar E; German collection of microorganisms) were used. These samples were prepared by adding various amounts of the reference material to the urine of patients that had been tested negative for CT before, resulting in concentrations of 0.03–195 inclusion-forming units (IFU)/mL of native urine. Concentrations chosen for spiking were generated randomly, based on published data on CT concentration in female FVU ranging from 3.5 to 5×10⁵ elementary body (EB)/mL (mean 470 EB/mL)¹⁹ and assuming 1 IFU corresponds to approximately 300 EB.²⁰ However, it should be noted that the relationship of infectivity and EB counts varies among the different serotypes.²⁰ Inclusion of spiked

samples was required to obtain sufficient numbers of CT-positive samples for the analysis of pooled urine samples, because the positivity rate in samples collected for screening was unexpectedly low.

All urine samples were divided into three parts for testing with the PelvoCheck CT/NG test, COBAS TaqMan CT Test V.2.0 and Abbott RealTime CT/NG. For the PelvoCheck CT/NG analysis, 3 mL of urine was transferred into specific collection tubes (PelvoCheck Collection kit SAFE) and stored at 0–8°C. These collection tubes contain a urine stabilising solution (under vacuum) allowing transfer of a defined volume of urine from the primary collection vessel. Another 1.5 mL aliquot of the native urine sample was stored at 0–8°C for the Roche COBAS TaqMan CT Test V.2.0 without adding any further stabilising agents. For the Abbott RealTime CT/NG test, 3 mL of urine was transferred to a multi-Collect Specimen Collection kit (Abbott) and stored at –20°C.

Pooled samples: 107 pools (52 negative and 55 positive) were analysed by PelvoCheck CT/NG. Negative pools consisted of equal volumes of five different urine samples that were negative by both PelvoCheck CT/NG and Roche COBAS TaqMan CT Test V.2.0, if tested individually. Positive pools consisted of equal volumes of one sample that tested CT positive by both PelvoCheck CT/NG and COBAS TaqMan CT Test V.2.0, and four samples with negative results in both tests. Twenty-five positive pools contained samples spiked with CT-positive reference material. To generate a single urine pool, 200 µL of each individual sample was applied. Consequently, the volume was lower than for the individual testing performed with 250 µL.

DNA extraction and amplification

porA PCR: Urine samples were analysed for NG using a PCR test targeting the *porA* pseudogene, according to the publication by Whiley *et al.*²¹ DNA was isolated from the urine, using the protocol for urine specimens preparation of the COBAS Amplicor CT/NG test (Roche). Five hundred microlitres of urine were combined with the same volume of CT/NG urine wash and incubated for 15 min at room temperature. After centrifugation at 12 500g for 5 min, the sediment was resuspended in 250 µL lysis reagent, again incubated at room temperature for 15 min and then combined with 250 µL of diluent. The sample was again centrifuged at 12 500g for 10 min and 10 µL supernatant was used for amplification on a LightCycler 1.5 (Roche). The DNA extract was combined with 12 µL 2× Quanti Tect Sybr Green Master mix (Qiagen), containing Hotstar Taq DNA polymerase, dNTPs and MgCl₂. In addition, a 2 µL primer mix containing papF (CGGTTTCCGTGCGTTACG) and papR (CTGGTTTCATCTGACTTTCCA) was added (final concentration of 0.5 µmol/L), as well as 2 µL sterile PCR grade water. The following temperature profile was used for amplification: initial activation of Hotstar Taq

DNA polymerase at 95°C for 15 min followed by 50 cycles of 94°C for 15 s, 55°C for 10 s and 72°C for 15 s. After amplification, the specificity of PCR products was verified by melting curve analysis.

PelvoCheck CT/NG test: Isolation of DNA from urine samples and processing of CT and NG were performed according to the instructions of the manufacturer. Stabilised urine of 250 µL (individual samples) or 1.0 mL (pooled samples consisting of five individual samples) was treated by using the oCheck Extraction Kit (Greiner Bio-One GmbH). The purified bacterial and human genomic DNAs were amplified with the PelvoCheck CT/NG ready-to-use Master Mix containing specific primer targeting a fragment of the 16S rRNA gene specific for CT and NG, and a fragment of the single-copy gene ADAT1 (sample control for the monitoring of sampling and DNA extraction). In the same reaction, an internal control template present in the Master Mix was amplified to monitor the PCR performance (PCR control). The amplification reaction was performed on an Applied Biosystems GeneAmp PCR System 9700. The specific PCR products, labelled with fluorescent dyes during the PCR reaction, were hybridised by mixing with the PelvoCheck CT/NG hybridisation buffer, under room temperature conditions, to the chip, with specific probes attached to the surface. The hybridisation efficiency is monitored by an internal control (hybridisation control). After removal of non-bound DNA by two washing steps, the PelvoCheck CT/NG chip was scanned, analysed and evaluated automatically by using the CheckScanner and the CheckReport Software.

Roche COBAS TaqMan CT Test V.2.0: Isolation of DNA from urine samples and amplification of CT was performed according to the manufacturer's instructions. The PCR test targets both the cryptic plasmid and the chromosomal *omp1* gene and was performed on a TaqMan 48 analyser (Roche).

Abbott RealTime CT/NG: Isolation of DNA from urine samples and amplification of CT and NG was performed according to the manufacturer's instructions. The PCR test targets two different regions of the CT cryptic plasmid and chromosomal *opa* gene sequences of NG and was performed on the Abbott m2000 platform.

RESULTS

The procedure of testing samples for NG and CT with the different PCR assays is shown in [figure 1](#). Five of 120 pretested urine specimens for NG analysis were excluded from the evaluation due to ambiguous identity or the possibility of contamination. In addition, 22 of 1672 samples for CT testing were excluded for the following reasons: ambiguous identity (n=10), aliquots missing for required tests (n=5), sample leaking (n=3), sample mixed up (n=2) and sample collection incorrect (n=2).

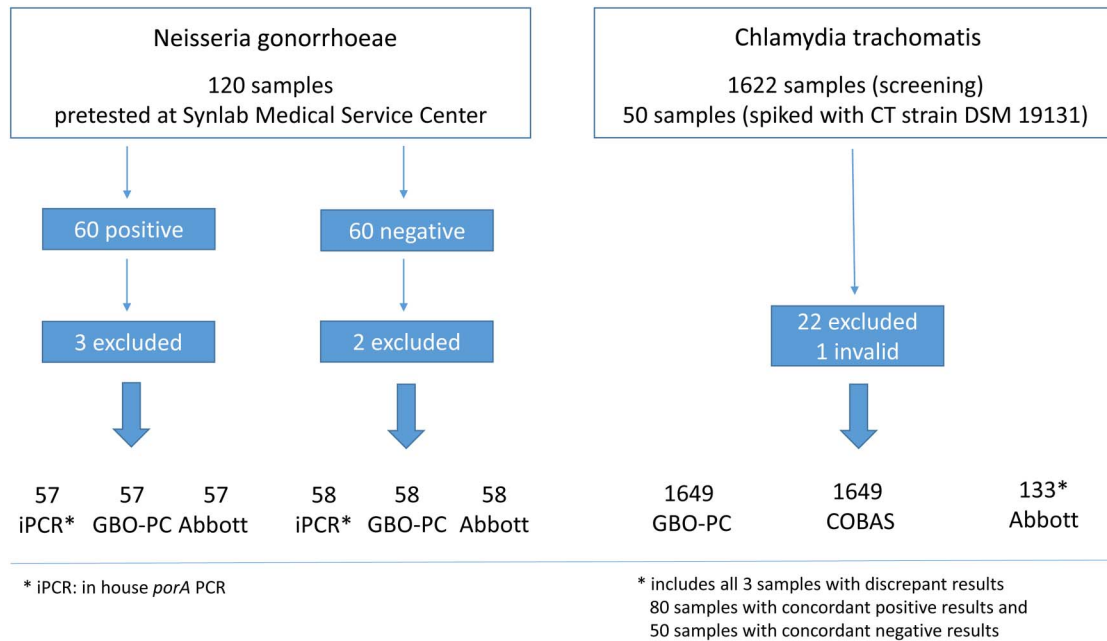


Figure 1 Flow chart of the testing procedure.

Evaluation of gonococcal DNA detection by PelvoCheck CT/NG

One hundred and fifteen urine samples pretested at Synlab Medical Service Center GmbH Weiden, Germany, using an *opa*-based in-house PCR assay (57 positive samples and 58 negative samples), were analysed for gonococcal DNA by both PelvoCheck CT/NG and another in-house PCR test based on the *porA* pseudogene. The results are summarised in table 1. Concordantly positive and concordantly negative results were obtained in 54 and 57 samples, respectively. Three samples were PelvoCheck CT/NG positive but negative by *porA* PCR, and one sample was positive by the *porA* PCR and negative by PelvoCheck CT/NG (table 1). Consequently, the positive and negative agreement was 98.2% (54/55) and 95.0% (57/60), respectively, and total agreement was 96.5% (111/115).

To verify the test results, in particular to resolve discrepant findings of the two PCR tests, the 115 samples were analysed with a second comparative assay (Abbott RealTime CT/NG). All 111 concordant results were confirmed with the second assay. Two samples with

PelvoCheck CT/NG positive/*porA* PCR negative results as well as one sample with a PelvoCheck CT/NG negative/*porA* PCR positive result, tested positive by Abbott RealTime CT/NG. Another sample with a positive PelvoCheck CT/NG test but negative *porA* PCR test was negative by Abbott RealTime CT/NG (table 2). Considering the result of Abbott RealTime assay as the decisive result in case of discrepancies between PelvoCheck CT/NG and *porA* PCR, 57 and 58 samples were defined as true positive and true negative, respectively. Consequently, there is one false negative and one false-positive PelvoCheck CT/NG test, resulting in 98.2% (56/57) sensitivity and 98.3% (57/58) specificity. The sensitivity and specificity of the *porA* PCR is 96.5% (55/57) and 100% (58/58).

All four samples with discrepant results in PelvoCheck CT/NG and *porA* PCR initially tested positive at the Synlab Medical Service Center GmbH, Weiden (table 2). Thus, the sample that was positive by PelvoCheck CT/NG but negative by both *porA* PCR and the Abbott

Table 1 Comparison of gonococcal PCR results of in-house PCR (*porA*) and PelvoCheck *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG)

N=115	<i>porA</i> PCR positive	<i>porA</i> PCR negative
PelvoCheck CT/NG positive	54	3
PelvoCheck CT/NG negative	1	57

Table 2 Results of the methods used for the analysis of samples with discrepant findings in PelvoCheck *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) and *porA* PCR

Sample	In-house PCR (<i>opa</i>)	PelvoCheck CT/NG	In-house PCR (<i>porA</i>)	Abbott RealTime CT/NG
1	+	+	-	+
2	+	+	-	-
3	+	+	-	+
4	+	-	+	+

RealTime CT/NG assay, might in fact represent a true-positive result.

To calculate predictive values for NG detection by PelvoCheck CT/NG, the results of the tested panel of 58 negatives and 57 positives are inappropriate, as they do not reflect the real prevalence. On the basis of the data from the Robert Koch Institute, the German National Institute for Surveillance and Prevention of Diseases, the prevalence of NG is assumed to be 3.7% in risk populations and 0.1% in sexually active individuals of the general population.^{22 23} In addition, calculating the positive predictive value (PPV) for NG in a low prevalence population requires testing of a larger number of samples to determine the number of false positives more precisely. Therefore, we also considered the NG results of 1622 urine samples collected for CT screening by PelvoCheck CT/NG, these were negative in all cases. As only a subset of 133 samples were tested with Abbott RealTime CT/NG as a comparative test, we cannot definitely exclude false negatives, however, the Chlamydia screening samples came from a low-risk population where few, if any, NG cases would be expected. When adding these 1622 negatives to the 58 true negatives from the NG test panel, the specificity of PelvoCheck CT/NG for NG detection would increase to 99.9% (1679/1680) and the PPV would be 97.3% and 49.5% in populations with prevalence rates of 3.7% and 0.1%, respectively. The negative predictive value (NPV) would be 99.9% in both populations.

Evaluation of CT DNA detection by PelvoCheck CT/NG

A total of 1650 samples were analysed for CT DNA by both the PelvoCheck CT/NG test and Roche COBAS TaqMan CT Test V.2.0. One sample was not considered for evaluation, as no valid result was obtained by the Roche COBAS TaqMan CT Test V.2.0. Thus, results of 1649 samples were used for the evaluation. Of these, 1599 were from female probands. As only 33 samples (2.1%) were positive, another 50 samples spiked with various amounts of CT strain DSM-19131 reflecting concentrations that occur in reality¹⁹ were included in order to obtain a number of 55 CT-positive samples required for the analysis of pooled urine samples (see below). Results of CT testing are summarised in table 3.

Table 3 Comparison of CT results obtained by Roche COBAS TaqMan CT V.2.0 and PelvoCheck *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) tests

	COBAS TaqMan CT Test V.2.0 CT positive	COBAS TaqMan CT Test V.2.0 negative
N=1649		
PelvoCheck CT/ NG positive	80	2
PelvoCheck CT/ NG negative	1	1566

Concordantly positive and concordantly negative results were obtained from 1646 samples (80 positive and 1566 negative samples, respectively). Two samples were PelvoCheck CT/NG positive, but Roche COBAS TaqMan CT Test V.2.0 negative (one of them was spiked with CT DNA). Another sample also spiked with CT-positive reference material was Roche COBAS TaqMan CT Test V.2.0 positive and PelvoCheck CT/NG negative (table 3). These results correspond to a positive and negative agreement of 98.8% (80/81) and 99.9% (1566/1568), respectively. The total agreement was 99.8% (1646/1649). To evaluate discordant results, Abbott RealTime CT/NG was used as a second comparative test. A total of 133 samples including the three samples with discordant results, and an additional 130 samples with concordant results of PelvoCheck CT/NG and Roche COBAS TaqMan CT Test V.2.0 (80 positives and 50 negatives) were tested. All concordant results were confirmed by the Abbott RealTime CT/NG test. The three samples with discordant results were positively tested by the Abbott RealTime CT/NG assay (table 4). Thus, regarding the Abbott RealTime CT/NG test result as deciding in case of discordant results, 83 positive and 1566 negative samples were defined. Consequently, no false-positive results were obtained using both PelvoCheck CT/NG and Roche COBAS TaqMan CT Test V.2.0 (specificity 100%, PPV 100%). The PelvoCheck CT/NG test detected 82/83 CT-positive samples, resulting in a sensitivity of 98.8% and NPV of 99.9%, comparable to the Roche COBAS TaqMan CT Test V.2.0, with 97.6% sensitivity and NPV of 99.9%.

Evaluation of detection of CT DNA by PelvoCheck CT/NG in pooled urine samples

As Chlamydia screening in Germany currently allows testing of pooled urine samples derived from up to five patients, the performance of the PelvoCheck CT/NG test was also examined for pooled urine samples. A total of 52 negative and 55 positive pools was analysed. Results are summarised in table 5. All 52 pools being composed of only negative samples were negatively tested by PelvoCheck CT/NG (specificity 100%). Negative results were also detected in five of 55 pools containing one sample that was positive when tested individually. Among the five false negative pools, four contained spiked urine samples, each with a low final chlamydia concentration (0.17–0.46 IFU/mL stabilised urine). Accordingly, the sensitivity for testing pooled urine samples is 50/55 (90.9%).

DISCUSSION

NAATs are the most sensitive tests to detect CT and NG. Thus, they are generally used as diagnostic tests in Chlamydia screening program, to reduce the incidence of infection and complications.²⁴ The German Chlamydia Screening Program also stipulates testing by NAATs that must be performed on FVU samples. Owing

Table 4 Results of the methods used for the analysis of samples with discrepant findings in PelvoCheck *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) and Roche COBAS TaqMan CT V.2.0

Sample	PelvoCheck CT/NG	COBAS TaqMan CT Test V.2.0	Abbott RealTime CT/NG	Type of sample	CT concentration (inclusion-forming units /mL)
1	+	–	+	Patient sample	Unknown
2	+	–	+	Spiked sample	0.35
3	–	+	+	Spiked sample	0.0375

to the same mode of transmission, co-infections with CT and NG may occur, accounting for up to 20% in some populations.²³ For this reason, many commercial tests are designed as duplex assays simultaneously detecting both pathogens. We evaluated the performance of the PelvoCheck CT/NG test kit, a new Multiplex-PCR test for detecting CT and NG in FVU, by comparing it with other PCR-based assays. Test results for NG obtained with PelvoCheck CT/NG agree to a large extent with those of an in-house *porA*-based PCR test (111/115, 96.5%) as well as with an Abbott RealTime CT/NG test system (113/115, 98.2%). Considering the Abbott RealTime CT/NG assay as a reference test, the PelvoCheck CT/NG test identified two NG-positive sample that were negative in the *porA* PCR, resulting in a slightly higher sensitivity (98.2% vs 96.5%). The discordant findings may result from samples with borderline NG concentration and higher analytical sensitivity of the PelvoCheck CT/NG test, or may relate to *porA* sequence variations recently described for a gonococcal strain isolated in Australia.¹⁵ However, one PelvoCheck CT/NG-positive sample was negative by both Abbott RealTime assay and *porA* PCR, resulting in a specificity of PelvoCheck CT/NG of 98.3% (57/58) that appears to be lower than for the *porA* PCR assay (100%). However, that sample could in fact be true positive, as it was initially tested NG positive at Synlab Medical Service Center GmbH. Possibly, the sample was only detectable with the PelvoCheck CT/NG test because of a higher analytical sensitivity compared to Abbott RealTime CT/NG and the in-house *porA* assay. Predictive values for NG detection by PelvoCheck CT/NG were estimated after adding NG test results of urine samples collected for Chlamydia screening because the NG test panel of 57 positives and 58 negatives does not reflect the real prevalence, and more accurate determination of false positives requires testing of a large number of samples from

a population with low prevalence. As none of the Chlamydia screening samples were NG positive, the specificity increases to 99.9%, but even then, the PPV of PelvoCheck CT/NG will be around 50% in a population with 0.1% NG prevalence. Similarly, low PPVs of NG detection were also described for other commercially available Food and Drug Administration (FDA)-approved NAATs when applied in low prevalence populations,²⁵ implying the necessity to confirm positive test results. However, confirmation of positive NAATs was reported to be difficult if the confirmatory test has a lower sensitivity than the initially used test, especially in samples containing low copy numbers of the target sequence;²⁶ that is, positive NAAT results not confirmed by another NAAT are not inevitably false positives, but may also represent false negative results of the confirmatory test.

High agreement of test results obtained with the PelvoCheck CT/NG test and Roche COBAS TaqMan CT Test V.2.0 or Abbott RealTime CT/NG, was also seen in CT testing, with concordant results in 99.8% and 99.2%, respectively. The prevalence of CT in screening samples was unexpectedly low (2.1%). These samples were collected in Southern Bavaria, a region where CT infection appears to be less prevalent. Indeed, a recently published CT sentinel report from the Robert Koch Institute indicates regional differences of CT infection rates ranging between 2.8% and 7.2%, with a rather low rate of 3.2% in Bavaria.²⁷ Owing to the low positive rate in screening samples, we included artificially positive samples generated by spiking negative urine specimens with defined amounts of genotype E EBs.

When regarding Abbott RealTime CT/NG as a reference test, the specificity of the PelvoCheck CT/NG test and Roche COBAS TaqMan CT Test V.2.0 was 100% in each case, but the sensitivity of PelvoCheckCT/NG for CT DNA appears to be slightly higher than for Roche COBAS TaqMan CT Test V.2.0 (98.8% vs 97.6%).

In a study from Sweden evaluating the Roche COBAS TaqMan CT Test V.2.0 test, high sensitivity and specificity of 100% and 99.8% were reported.²⁸ Another study comparing Abbott RealTime CT/NG with Roche COBAS TaqMan CT Test V.2.0 and Hologic Aptima Combo 2 for CT/NG confirmed the high specificity of Roche COBAS TaqMan CT Test V.2.0 but reported significantly lower sensitivity to detect CT in urine samples compared to the Hologic Aptima Combo 2 for CT/NG and Abbott RealTime CT/NG assay.²⁹ The reduced sensitivity may depend on less-efficient sample preparation, as in that

Table 5 Analysis of pooled urine samples by the PelvoCheck *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) test

Pool composition	Number	Pool result CT positive	Pool result CT negative
Positive (1), negative (4)	55	50	5
Negative (5)	52	0	52

study half of the false-negative Roche COBAS TaqMan CT Test V.2.0 samples became positive when using the DNA preparation from the Abbott m2000 system, for amplification.²⁹

In our study, two samples with discrepant results (one PelvoCheck CT/NG positive and Roche COBAS TaqMan CT Test V.2.0 negative, and one Roche COBAS TaqMan CT Test V.2.0 positive and PelvoCheck CT/NG CT negative) represented spiked samples with low concentrations of chlamydia (<1 IFU/mL). According to Roche, the limit of detection (LOD) of the COBAS TaqMan CT Test V.2.0 is 1–2 IFU/mL, while the LOD of PelvoCheck CT/NG for individual samples is 0.3 IFU/mL (PelvoCheck CT/NG, instructions for use). Although other spiked samples with similarly low CT concentrations were positive in both assays, low target concentration may result in aliquots without any target due to stochastic distribution of target DNA (ie, assuming a target concentration of 10 copies/mL, not all 100 µL aliquots will contain one copy of target DNA). Thus, in samples with low target concentrations, false negative results may occur with a probability depending on the analytical sensitivity of the method, including preanalytical sample preparation.

The German Chlamydia Screening Program allows laboratories to combine up to five samples from different persons for one test. Therefore, we evaluated the performance of the PelvoCheck CT/NG test for pooled urine samples. No invalid or false-positive results were observed in pooled samples. However, in 5 of 55 positive pools, CT DNA was not detected (sensitivity 90.9%), probably due to low target concentration below the LOD of the PelvoCheck CT/NG test, as a consequence of pooling-associated dilution. However, this issue represents a general problem associated with the procedure of testing pooled samples rather than a test-specific characteristic. In agreement with our study, loss of sensitivity in testing pooled urine samples has also been described in several other studies. For instance, in a study from England, it has been reported that 8.8% and 8.2% of pools containing one CT-positive sample were not detected by ProbeTec (BD) or COBAS Amplicor (Roche), respectively.⁸

In conclusion, the PelvoCheck CT/NG test is a suitable test method for the detection of CT and NG in female FVU samples, with sensitivity and specificity comparable with other FDA approved CT/NG NAATs. The test may also be used for pooled urine samples. However, one should take into consideration that sensitivity is reduced by pooling, and that extensive handling of samples may increase the risk of contamination and requires effective quality control measures.¹¹

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Contributors TM was involved in study design, data analysis and writing of the manuscript. CK was responsible for sample collection, data analysis and review of the manuscript. AK participated in sample collection, supervision of data analysis and review of the manuscript. KH was involved in study design, sample collection, data analysis and review of the manuscript; RK was responsible for study design, supervision of data analysis and review of the manuscript.

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Competing interests RK, AK and KH are employed at Greiner-Bio-One GmbH.

Patient consent Obtained.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Detailed test results of all specimens are available by emailing th.meyer@uke.de.

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