Evaluation of the New Test VERSANT CT/GC DNA 1.0 Assay for the Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Urine Specimens

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Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (GC) are the two most common sexually transmitted bacterial infections in developed countries. The purpose of the present study was evaluating a new system for CT/GC detection in urine specimens. A total of 700 urine specimens were obtained from patients attending the STD Outpatients Clinic of St. Orsola University Hospital, Bologna, Italy. Samples were tested by VERSANT[®] CT/GC DNA 1.0 Assay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY), a multiplex Real-Time PCR assay, for simultaneous CT/GC detection. Results obtained by VERSANT assay were compared with those obtained

by culturing genital secretions of the same patients. Moreover, urine specimens testing positive in VERSANT assay were retested by in-house PCR assays, used as confirmatory tests. VERSANT[®] CT/GC DNA 1.0 Assay performed with 99.4% and 99.2% of specificity for GC and CT detection, respectively, whereas sensitivity was 100% both for CT and GC. Culture methods were 100% specific, but far less sensitive than VERSANT assay. VERSANT[®] CT/GC DNA 1.0 Assay demonstrated to be a highly sensitive and specific technique for CT/GC detection. J. Clin. Lab. Anal. 26:70–72, 2012.

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INTRODUCTION

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (GC) are the two most common sexually transmitted bacterial infections in developed countries. Regarding CT, up to 70% of infected women and 75% of infected men are asymptomatic, making screening of the disease necessary to identify and treat the infection (1).

Since the introduction of nucleic acid amplification tests (NAATs) for CT/GC detection in genital tract specimens, this approach has become the standard diagnostic method used in most laboratories. Moreover, urine has also been validated as an adequate specimen for diagnostic testing (2–4).

Although several commercial assays are available for performing nucleic acid amplifications tests, which are now recommended as the test of choice, the quest for new assays and new platforms is going on (4).

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Here, we report on the prospective evaluation of a new fully automated system for CT/GC detection in urine specimens. A total of 700 urine specimens were tested using VERSANT CT/GC DNA 1.0 Assay, in conjunction with the VERSANT kPCR Molecular System (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). Results obtained by VERSANT assay were compared with those obtained by culturing genital secretions of the same patients. Moreover, urine specimens testing positive in VERSANT assay were retested, using the same extraction, by in-house PCR assays, used here as confirmatory tests.

MATERIALS AND METHODS

We enrolled for the study 700 subjects attending the Sexually Transmitted Diseases (STD) Outpatients Clinic of the St. Orsola University Hospital in Bologna, Italy. Subjects were eligible for the study if they were sexually active, aged 18 or older and met one or more of the following criteria: seeking STD screening, seeking treatment for STD-related symptoms, or reported unsafe sex practice. Three specimens were collected from each subject: first, two urethral or endocervical (from men and women, respectively) swabs for the detection of CT and GC by culture, and then one urine sample.

VERSANT CT/GC DNA 1.0 Assay is a new fully automated qualitative in vitro nucleic acid amplification assay based on kPCR technology to be used in conjunction with the VERSANT kPCR Molecular System (Siemens Healthcare Diagnostics Inc.). Chlamydia Taqman[®] PCR primers and probes were designed to target the GenBank nucleic acid sequence of the 7.5-kb cryptic plasmid outside of 377 bp deletion (nvCT mutant). Neisseria Taqman[®] PCR primers and probes were designed to target the nucleic acid sequence of the multiple copy gene locus of *pivNG* gene.

GC was isolated in Thayer-Martin medium and identified by API NH assay (bioMérieux, Marcy l'Etoile, France).

CT was isolated in LLC-MK2 cells and identified by fluorescein-conjugated monoclonal antibody, as previously described (5).

Specimens testing positive in VERSANT CT/GC DNA 1.0 Assay were retested, using the same extraction, by in-house PCR assays; for CT the assay target was *omp1* (6–8), whereas for GC the target was *cpxB* gene, carried on both the chromosome and the 4.2-kb cryptic plasmid (9).

For CT detection, a Nested PCR was performed: the first product of 1,222 bp was amplified using CT90UF (5'-GGACATCTTGCTCTGGCTTTAACT-3') and modified NRO (5'-GTCTCAACTGTAAGTGCATTTT-3'). In the second PCR step, amplifying a 1,075-bp fragment, primers SERO 1A (5'-ATGAAAAAACTCTTGAAA

TCGG-3') and modified NRI (5'-TACCGCAAGATTTTCTAGATTT-3') were used.

The PCR product for GC detection was 390 base pairs long and the following primer pair was used: HO1 (5'-GCTACGCATACCCGCGTTGC-3') and H03 (5'-CGAAGACG1TCGAGCAGACA-3').

In the present study, the results obtained by VERSANT assay and culture were classified as true positive, false positive, false negative, and true negative, as follows: if a result from culture was negative, but VERSANT assay was positive and confirmed positive by the in-house PCR assay, the negative result was designated as a false negative. Similarly, a positive result from VERSANT assay was designated as a false positive if both the culture and the in-house PCR assay were negative.

RESULTS

CT and GC results are summarized in Tables 1 and 2, respectively.

VERSANT CT/GC DNA 1.0 Assay performed with 99.4% and 99.2% of specificity for GC and CT detection, respectively. Sensitivity of VERSANT assay was 100%, both for CT and GC. Both culture methods were 100% specific, but far less sensitive than VERSANT assay.

In particular, GC culture was 89.2% sensitive, whereas CT culture was only 70.5% sensitive.

TABLE 1. Comparative Performance of VERSANT CT/GC DNA 1.0 Assay and Culture for the Detection of *C. trachomatis* in Urine Specimens and Genital Secretions, Respectively

Parameter	VERSANT CT/GC DNA 1.0	<i>C. trachomatis</i> culture
No. of true positive	78	55
No. of false positive	5	0
No. of false negative	0	23
No. of true negative	617	622
% of sensitivity	100	70.5
% of specificity	99.2	100

TABLE 2. Comparative Performance of VERSANT CT/GC DNA 1.0 Assay and Culture for the Detection of *N. gonorrhoeae* in Urine Specimens and Genital Secretions, Respectively

Parameter	VERSANT CT/GC DNA 1.0	<i>N. gonorrhoeae</i> culture
No. of true positive	37	33
No. of false positive	4	0
No. of false negative	0	4
No. of true negative	659	663
% of Sensitivity	100	89.2
% of Specificity	99.4	100

DISCUSSION

Many studies have shown that conventional methods such as culture or enzyme immunoassay tests have a significantly lower sensitivity than NAATs for CT detection (10). Moreover, the possibility of testing urine specimens have made NAATs the recommended choice for screening programs, where noninvasive and convenient self-collection is preferred.

The ideal test for CT/GC detection should be highly sensitive, in order to identify all the infected subjects and to prevent both transmission to sexual partners and serious sequelae. However, also the specificity of a NAAT is critical, particularly when the test is used in a low-prevalence population (2).

Additional factors, other than analytical sensitivity and specificity, should be considered in evaluating automated systems for CT/GC detection. VERSANT system showed to be very easy to use, little labor intensive and it did not require any specimen processing before loading samples onto the VERSANT kPCR Molecular System. Moreover, the good quality of its DNA extracts allowed us to amplify *omp1* gene by Nested PCR.

Since chlamydial serovar typing is widely performed after PCR amplification and DNA sequencing of *omp1* gene, VERSANT system could be an intriguing aid in future CT typing and epidemiological studies.

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