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Advantages of Syndromic Diagnostics: Detection of the Pathogens Causing Urethritis/Cervicitis with the STI CNM Real-Time PCR Kit from Vitro S.A.

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Abstract

The STI CNM Real-Time PCR Kit from Vitro S.A. (Spain) demonstrates high sensitivity and specificity, is cost-effective, and can detect the three main etiological agents of urethritis/cervicitis in a single multiplex PCR. Sexually transmitted infections (STIs) are a significant public health problem and a significant burden of morbidity and mortality in hospitals. The World Health Organization (WHO) estimates the number of daily infections to be 1 million. Currently, the number of infections and antimicrobial-resistant strains is rising. A rapid and accurate etiologic diagnosis is critical to solving this problem. In this study, we compared the STI CNM Real-Time PCR Kit using the Xpert® CT/NG technique (Cepheid®, USA) as Gold Standard for the diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* and EasyNAT® MG (Ustar Biotechnologies (Hangzhou) Ltd., China) as Gold Standard for the diagnosis of *Mycoplasma genitalium* infection. Regarding *C. trachomatis* and *N. gonorrhoeae*, out of 200 samples, there was a match in 199 cases, with only one positive sample not being detected by the STI CNM Real-Time PCR Kit. This results in a sensitivity of 96% and a specificity of 100% for this kit. Diagnosing *M. genitalium* infection, out of 200 samples, the STI CNM Real-Time PCR Kit correctly detected all negative and positive samples, with 100% agreement compared to the reference technique. In summary, the STI assay has a very high sensitivity and specificity, comparable to other commercial diagnostic kits. Furthermore, it has the advantage of bundling the detection of the three main bacterial agents of urethritis/cervicitis, resulting in better cost efficiency.

Keywords: STI, syndromic diagnostics, *Mycoplasma genitalium*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*

Introduction

Sexually transmitted infections (STIs) represent a significant public health challenge due to their high prevalence and potential to cause serious short- and long-term consequences. More than 30 pathogens, including bacteria, viruses, and parasites, are transmitted primarily through sexual contact or vertical transmission (Jansen et al. 2020; WHO 2022). Among these, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium* are the most common bacterial STIs, each contributing to morbidity, reproductive complications, and facilitating the transmission of HIV (Jensen et al. 2022; Tuddenham et al. 2022).

The World Health Organization (WHO) estimates that 1 million people acquire a new STI daily, underscoring these infections' global burden (WHO 2021; Sinka 2024). While syndromic management has traditionally been the standard approach, relying on clinical symptoms to guide treatment (WHO 2003), this method has several limitations. Infections such as *C. trachomatis*, *N. gonorrhoeae*, and *M. genitalium* frequently present with asymptomatic or non-specific symptoms, particularly in women and HIV-positive populations, making syndromic management insufficient for accurate detection (Farfour et al. 2021). To address this, molecular diagnostic techniques, particularly nucleic acid amplification tests (NAATs) like real-time PCR and multiplex PCR, have gained prominence for their sensitivity, specificity, and ability to screen asymptomatic individuals at risk (Unemo et al. 2017; Levy et al. 2019; de Vries et al. 2019).

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C. trachomatis remains the most common bacterial STI globally, with young adults being particularly vulnerable (Lane and Decker 2016). *N. gonorrhoeae*, however, poses an additional challenge due to increasing antibiotic resistance, which threatens to render it untreatable shortly (Unemo et al. 2020). Likewise, *M. genitalium* is gaining recognition for its high prevalence and alarming resistance to macrolides, necessitating advanced diagnostic tools that also detect resistance mutations (Horner et al. 2019; Gnanadurai and Fifer 2020).

Molecular diagnostics, particularly NAATs, have revolutionized the detection of these pathogens. Techniques such as real-time PCR and multiplex PCR allow for the simultaneous detection of multiple STI pathogens in a single test, which is a major advance over traditional methods like bacterial culture or immunoassays. These molecular tests improve diagnostic accuracy, reduce turnaround times, and enable earlier treatment interventions (de Vries et al. 2019; Unemo et al. 2019). However, their cost remains a significant barrier in many settings, particularly in low-resource regions where STIs are highly prevalent (Saweri et al. 2019; Mallik et al. 2020).

While the high sensitivity and specificity of these tests offer significant benefits in terms of public health and long-term cost savings, the initial investment required for implementing molecular diagnostics can be prohibitive. Therefore, a thorough evaluation of the cost-benefit ratio is essential to determine the most appropriate diagnostic approach in various clinical settings. Multiplex PCR techniques offer several advantages over conventional PCR for the diagnosis of sexually transmitted infections (STIs). One of the main benefits is the ability to simultaneously detect multiple pathogens in a single assay, which significantly improves diagnostic efficiency, especially for complex infections where multiple causative agents may be involved. These molecular tests improve diagnostic accuracy, reduce turnaround times, and enable earlier treatment interventions (Ramanan et al. 2017; Dien Bard and McElvania 2020; Naeem et al. 2021). Studies have shown that multiplex PCR can deliver highly sensitive and specific results for common STI pathogens, such as *C. trachomatis*, *N. gonorrhoeae*, and *M. genitalium*, compared to traditional PCR methods, which usually target a single pathogen at a time (Medhi et al. 2024).

This study aims to compare the performance of the STI CNM Real-Time PCR Kit (Vitro S.A, Spain) in the diagnosis of *C. trachomatis*, *N. gonorrhoeae*, and *M. genitalium* by evaluating its sensitivity, specificity, and level of concordance with two other commercial methods: the Xpert® CT/NG Kit (Cepheid®, USA) and EasyNAT® MG (Ustar Biotechnologies (Hangzhou) Ltd., China).

Experimental

Materials and Methods

Two commercial RT-PCR kits were compared for the detection of *C. trachomatis*, *N. gonorrhoeae*, and *M. genitalium*, based on the criteria of the Clinical and Laboratory Standards Institute (CLSI 2008).

The study was conducted at the Infanta Elena Hospital in Huelva, Andalusia, Spain, in 2023. A total of 200 samples were collected from different anatomical sites from patients attending our hospital's infectious diseases clinic for the determination of *C. trachomatis*, *N. gonorrhoeae*, and *M. genitalium*. The samples were tested simultaneously using the different methods described.

The determinations were performed with the Xpert® CT/NG Kit; the commercial STI CNM Real-Time PCR Kit, determined on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems™, Thermo Fisher Scientific, Inc., USA) after extraction on a Nextractor® NX-48S (Genolution Inc., Republic of Korea); and the EasyNAT® MG on an EasyNAT® 2.0 device. The primers and probes of the Xpert® CT/NG Kit recognize chromosomal sequences of *C. trachomatis* (CT1: one target) and *N. gonorrhoeae* (NG2 and NG4: two different targets). The STI CNM Real-Time PCR Kit uses fluorescent primers and probes for the target genes Opal (*N. gonorrhoeae*), cryptic plasmid (*C. trachomatis*) and MgPa (*M. genitalium*). The Xpert® CT/NG Kit measures *C. trachomatis* and *N. gonorrhoeae* in a single step, while the STI CNM Real-Time PCR Kit also measures *M. genitalium*. The EasyNAT® MG only measures *M. genitalium* in a single step.

All tests were performed and analyzed according to the manufacturer's instructions. Relevant guidelines and regulations were followed for all methods. Hospital Infanta Elena Ethics Committee waived the need for ethical approval and informed consent.

Cohen's kappa coefficient and Spearman's correlation were used for the statistical analyses to assess the agreement level. Diagnostic test efficiency indicators (sensitivity, specificity, positive and negative predictive values) were also utilized. These indicators were introduced by Yerushalmy (1947) and are widely used today to evaluate the efficiency of a diagnostic test compared to a reference standard.

Results

A total of 200 samples were collected from 90 patients (14 women and 76 men), whose ages ranged from 18 to 79, with a mean age of 35 and a median age of 37 (Table SI).

Below are the results obtained with the different diagnostic techniques. Table I shows the results

Table I
Results obtained with Xpert CT/NG® and EasyNAT MG® from Ustar (MG).

	Chlamydia trachomatis		*Neisseria gonorrhoeae*		*Mycoplasma genitalium*	
	NEG	POS	NEG	POS	NEG	POS
Pharyngeal swab (34.0%)	65	3	64	4	65	3
Rectal swab (31.5%)	57	6	62	1	57	6
Urethral swab (25.0%)	44	6	48	2	48	2
Endocervical swab (5.5%)	10	1	11	0	11	0
Initial urine (4.0%)	8	0	8	0	0	8

obtained with the Xpert® CT/NG system for *C. trachomatis* and *N. gonorrhoeae*, a method of proven reliability (Xie et al. 2020; Cordioli et al. 2024). We consider this method to be the reference method as it has been recommended by the WHO (Jacobsson et al. 2018). In addition, the results for *M. genitalium* obtained with the EasyNAT® MG are shown.

A total of 41 samples (20.5%) tested positive. 8% were positive for *C. trachomatis*, 3.5% for *N. gonorrhoeae* and 9.5% for *M. genitalium*. One sample was positive for both *C. trachomatis* and *M. genitalium*. Regarding anatomical localization, *C. trachomatis* showed a higher prevalence in urethral and rectal swabs, while *N. gonorrhoeae* was more common in pharyngeal swabs. The remarkably high positivity rate (100%) for *M. genitalium* in urine is surprising and can be explained by the fact that in our hospital, this sample is only sent to the laboratory in patients with a high suspicion of persistent urethritis after *C. trachomatis*/*N. gonorrhoeae* had previously been excluded. Only one sample (*C. trachomatis*) tested positive in the endocervical smear, although the small number of samples of this type analyzed does not allow for conclusive results.

Table II
Concordance table between both techniques.
Below, statistical parameters are shown.

		Xpert® CT/NG	
		POS	NEG
STI CNM Real Time	POS	29	0
PCR Kit	NEG	1*	170
Sensitivity: 96%			
Specificity: 100%			
Positive predictive value: 100%			
Negative predictive value: 99%			
Kappa index: 0,98			
Standard error: 0,071			
C.I. 95%: 0,84–1,10			
Concordance: very good			

* Detailed analysis of this single discordant sample showed a slight amplification around cycle 39, suggesting that the sensitivity of the technique would be increased at a higher threshold.

Comparative results (Xpert® CT/NG vs. STI CNM Real-Time PCR Kit). Table II is a concordance table between the two techniques. As shown, the agreement between the two is almost complete, both for positive and negative samples. The only positive sample that the STI CNM Real Time PCR Kit failed to detect tested positive.

Comparative results (EasyNAT® MG vs. STI CNM Real-Time PCR Kit). Table III shows the results of the comparison between the two techniques used to detect *M. genitalium*. As shown, there is complete agreement between the two.

Discussion

Ever since the American CDC recommended it in 2014 (Papp et al. 2014), nucleic acid amplification techniques have gained significant popularity in STI diagnostics. These techniques are favored due to their high sensitivity, specificity, and quick turnaround time for results. Over the years, several diagnostic kits have been introduced, improving the accuracy of STI diagnosis, streamlining laboratory processes, and enhancing the management of these infections. Currently, numerous studies validate the diagnostic efficacy of

Table III
Concordance table between both techniques.
Below, statistical parameters are shown.

		EasyNAT® MG	
		POS	NEG
STI CNM Real Time	POS	19	0
PCR Kit	NEG	0	181
Sensitivity: 100% (IC: 90,6–100%)			
Specificity: 100% (IC: 96,9–100%)			
Positive predictive value: 100% (IC: 83,1–100%)			
Negative predictive value: 100% (IC: 98,3–100%)			
Kappa index: 1			
Standard error: 0,02			
C.I. 95%: 0,93–1,01			
Concordance: excellent			

many of these tools (Bristow et al. 2017; Levy et al. 2019; Herrmann and Malm 2021; Tuddenham et al. 2022. These studies consistently demonstrate sensitivity and specificity results that are sufficiently high to warrant their routine use, replacing traditional culture and serology-based systems.

The efficacy of molecular techniques for diagnosing CT and NG has been extensively tested, and numerous commercial solutions offer high sensitivity and specificity both in the genital (Papp et al. 2014; Caruso et al. 2021) and extragenital infection (Cosentino et al. 2017; Cordioli et al. 2024). Our study aligns with this growing trend by showcasing that the STI CNM Real-Time PCR Kit from Vitro S.A. exhibits comparable sensitivity and specificity to other widely used techniques, such as the Xpert® CT/NG Kit, for diagnosing *C. trachomatis* and *N. gonorrhoeae* (Xie et al. 2020; Cordioli et al. 2024).

M. genitalium is accountable for a significant proportion of non-chlamydial and non-gonococcal urethritis in men, ranging from 10% to 35%, and cervicitis in women, ranging from 10% to 25% (Horner et al. 2019; Gnanadurai and Fifer 2020). In the past, identifying infections caused by this microorganism was challenging due to the difficulties associated with isolating it in culture. However, with the introduction of molecular techniques, the diagnosis of *M. genitalium* infections has improved. The European Academy of Dermatology and Venereology now recommends nucleic acid amplification techniques as the most effective diagnostic method and emphasizes the importance of considering this microorganism in the differential diagnosis of urethritis and cervicitis (Jensen et al. 2022). Despite this recommendation, limited commercial techniques are available that include *M. genitalium* in their diagnostic panels.

Nevertheless, the approval of the first molecular technique for its detection by the FDA (Shipitsyna and Unemo 2020) has led to several studies demonstrating the high sensitivity and specificity of these techniques (Lee et al. 2012; Le Roy et al. 2012; 2014; Vandepitte et al. 2014; Kirkconnell et al. 2019; Shipitsyna and Unemo 2020; Salazar and García 2022). In our study, we compared the STI CNM Real-Time PCR Kit from Vitro S.A. with the EasyNAT® MG from Ustar Biotechnologies (Hangzhou) Ltd. and found a strong agreement between both techniques, as well as high sensitivity and specificity. Therefore, like the recommendations for *C. trachomatis* and *N. gonorrhoeae*, we support the routine use of the STI CNM Real-Time PCR Kit in clinical laboratories.

However, the leading interest for the clinical laboratory in the STI assay from Vitro S.A. is not solely based on its commendable sensitivity and specificity. Instead, its value lies in identifying the three primary causative agents of urethritis/cervicitis through a single multiplex PCR reaction. This feature makes it an ideal candidate for integration into syndromic diagnostic strategies, which have proven to be cost-effective by enhancing patient management, optimizing antibiotic usage, preventing new infections, and streamlining overall laboratory operations (Ramanan et al. 2017; Dumkow et al. 2021; Karellis et al. 2022). Furthermore, in our laboratory, the implementation of this kit has resulted in a remarkable 76.5% reduction in economic costs for these analyses, reducing the expense from €68 to a mere €16 per test, which underscores its potential for improving cost-effectiveness in STI diagnostics.

The main limitation of our study was that it was carried out on routine clinical samples received in our laboratory without any specific recruitment of patients beyond selecting those with a suspected diagnosis of STI. This also meant that we did not reach a sufficiently high number of samples of each type analyzed. Therefore, although our results are in line with those of previous studies, which indicate that there are no significant differences in sensitivity or specificity based on the anatomical location of the sample (Bristow et al. 2017; Jansen et al. 2020; Herrmann and Malm 2021), it is essential to exercise caution when concluding due to the limited number of positive cases in specific anatomical locations.

To summarize, the STI CNM Real-Time PCR Kit developed by Vitro S.A. proves to be extremely valuable in identifying *N. gonorrhoeae*, *C. trachomatis*, and *M. genitalium* in various clinical samples, including urine, urethral, rectal, vaginal, endocervical and pharyngeal swabs. One of its key advantages is adopting a syndromic diagnostic approach, streamlining the sample processing procedure in the laboratory, expediting result delivery, and optimizing resource utilization. With its remarkable sensitivity and specificity, this kit is highly recommended for routine use in clinical laboratories as it consistently provides reliable results, thereby facilitating the effective management of sexually transmitted infections. Moreover, the kit's capability to simultaneously detect the three primary causative agents of urethritis/cervicitis through a single multiplex PCR reaction, coupled with the substantial reduction in economic costs observed in our laboratory, further underscores its practicality and convenience in the clinical setting.

Availability of data and material

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethical statement

The need for ethical approval and informed consent was waived by Hospital Infanta Elena Ethics Committe.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Miguel Ángel Castaño López, Inmaculada García Borrero and

Josefa Vazquez Medel. The first draft of the manuscript was written by Héctor Toledo Porteros and Alberto De La Iglesia Salgado. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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Supplementary materials are available on the journal's website.