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Original

# Usefulness of a novel multiplex real-time PCR assay for the diagnosis of sexually-transmitted infections

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ABSTRACT

*Introduction:* Sexually transmitted infections (STI) are currently on the increase worldwide. New molecular tools have been developed in the past few years in order to improve their diagnosis. An evaluation was carried out using a new commercially available real-time PCR assay, Anyplex™ II STI-7 (Seegene, Seoul, Korea), which detects seven major pathogens in a single reaction – *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* – and compared with conventional methods performed in our laboratory.
*Materials and methods:* Two different populations were included, and 267 specimens from different sites of infection (urines, endocervical swabs, rectal swabs, vaginal swabs, urethral swabs and one inguinal adenopathy) were processed for both methods.
*Results:* The parameters of clinical performance were calculated for *C. trachomatis, N. gonorrhoeae, and T. vaginalis*, and the assay achieved sensitivities (SE) from 93.94% to 100%, and specificities (SP) from 96.55% to 100%, with negative predictive values (NPV) from 93.33% to 98.85%, and positive predictive values (PPV) from 96.88% to 100%, with a very good agreement (kappa index from 0.88 to 1).
*Conclusions:* Anyplex™ II STI-7 is a good tool for the reliable diagnosis of STI. Its ease of use and processing allows it to be incorporated into the day to day laboratory work.



## Utilidad de un nuevo ensayo de PCR multiplex para el diagnóstico de las infecciones de transmisión sexual

*Palabras clave:*
Infecciones de transmisión sexual
PCR multiplex
Real-time PCR
*Neisseria gonorrhoeae*
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RESUMEN

*Introducción:* Las infecciones de transmisión sexual (ITS) son actualmente un problema de salud pública en todo el mundo debido al aumento que han experimentado en los últimos años que implica el desarrollo de nuevas herramientas moleculares para mejorar su diagnóstico. Se ha comparado el nuevo ensayo de PCR en tiempo real, Anyplex™ II STI-7 (Seegene, Seúl, Corea) que detecta los siete microorganismos implicados en las ITS – *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum*, and *Ureaplasma parvum* – en una sola reacción, con los métodos convencionales utilizados en nuestro laboratorio.

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*Métodos:* Se incluyeron dos tipos de poblaciones, obteniéndose 267 muestras de diferentes lugares de infección (orines, exudados endocervicales, frotis rectales, frotis vaginales, exudados uretrales y una adenopatía inguinal) que fueron procesadas por ambas metodologías.
*Resultados:* Las sensibilidades, especificidades y valores predictivos fueron analizados para *C. trachomatis, N. gonorrhoeae* y *T. vaginalis*, alcanzando sensibilidades (SE) de 93,94% a 100%, especificidades (SP) de 96,55% a 100%, valor predictivo negativo (NPV) entre 93,33% y el 98,85% y valores predictivos positivos (PPV) de 96.88% a 100% con muy buena correlación (índice kappa de 0.88 a 1).
*Conclusiones:* AnyplexTM II STI-7 es una buena herramienta para el diagnóstico seguro de las ITS. La facilidad de uso y procesamiento permite su incorporación en el trabajo del día a día del laboratorio.


## Introduction

Microbiological diagnosis of syndromes that can be caused by multiple pathogens, such as (STIs) may be challenging. Therefore, methods able to detect multiple microorganisms in a clinical specimen at the same time are essential. The need for the development of reliable, affordable, and effective commercial assays for the management of the syndromic diagnosis of STIs is rising, given their high prevalence and their increasing burden worldwide.[1] In 2008, the WHO estimated the total number of incident cases of the four main STIs in adults comprising ages between 15 and 49 years to be 498.9 million. Among them, 105.7 million corresponded to *Chlamydia trachomatis*, 106.1 million to *Neisseria gonorrhoeae*, 10.6 million to *Treponema pallidum*, and 276.4 million to *Trichomonas vaginalis*.[2] More than 30 different bacteria, viruses and parasites can potentially be transmitted through sexual contact, which makes the choice of diagnostic assays a difficult task. A selection of the most relevant pathogens according to local prevalence rates should be targeted.

Conventional diagnostic assays, such as culture and antigen detection assays, lack sensitivity, require viable organisms and thus special shipment conditions and, sometimes, invasive sampling.[3] As nucleic acid amplification tests (NAATs) allow us to overcome some of these limitations, several molecular diagnostic assays have recently been commercialized to assist the syndromic diagnosis of STIs. Nowadays, NAATs are flexible and easy to use. In addition, their implicit multiplexing capacity allows for the detection of multiple pathogens in a single sample and, therefore, their implementation in the clinical microbiology laboratory is increasing. The rising prevalence of STIs reported worldwide may be related, at least in part, to the use of improved diagnosis through the use of molecular technologies.[2]

The detection of multiple pathogens is achieved by NAATs thanks to its ability of multiplexing different assays.[4] Several molecular tests are commercially available for the diagnosis of STIs detecting a number of microorganisms ranging from two (i.e. Abbott RealTi*me*TM CT/NG, Abbott Molecular Inc., Des Plaines, IL), to 18 (i.e. CLART® STIs A&B, Genomica S.A.U., Madrid, Spain), thereby simplifying the diagnostic workflow, reducing the hands-on-time, as well as associated costs. Recently, a rapid test that detects *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in one step and in 90 minutes has been developed for the GeneXpert systems (Cepheid, Sunnyvale, CA). This constitutes the first step toward a highly reliable point-of-care test in the field of STIs.[5] Molecular tools have a high sensitivity and specificity for the diagnosis of the most prevalent STIs, and offer the possibility of using non-invasive specimens.[6,7]

In this study we have evaluated the usefulness of AnyplexTM II STI-7 (Seegene, Seoul, Korea), both for screening and diagnosis of STI. This real-time PCR assay detects seven major pathogens that cause STIs – *Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum*, and *Ureaplasma parvum* – in a single reaction.

## Material and methods

### *Study design*

This was a retrospective cross-sectional observational study designed for the clinical evaluation of the commercial AnyplexTM II STI-7 molecular assay in comparison with conventional diagnostic assays and other molecular assays used for the routine diagnosis of STI at the Microbiology Department of the "Germans Trias i Pujol" University Hospital (HUGTIP, Badalona, Spain). This study was approved by the Ethics Committee at our institution.

### *Study population and clinical samples*

Two different study populations were included in the present study. Group 1 included 234 individuals attended at the emergency room, the urology and gynecological departments at HUGTIP, primary care centers seeking medical care, as well as young adults (≤25 years old) suspected of having an STI and recruited for *C. trachomatis* prevalence studies at sexual and reproductive health centers. After routine microbiological diagnosis (culture and Abbott RealTimeTM CT/NG), residual sample volume was stored at −20 °C until used for the evaluation of the AnyplexTM II STI-7 assay. Group 2 included 33 HIV-negative MSM having high-risk sexual practices that were periodically screened for STIs at the BCN Checkpoint center (a community-based detection center of HIV and other STIs). After being tested by the Abbott RealTime CT/NG PCR Assay, residual sample volume was stored at −20 °C until used for this evaluation study.

Clinical specimens included 105 first-void urines, 50 rectal swabs, 18 urethral swabs, and three inguinal lymphadenopathy samples from patients suspected for lymphogranuloma venereum (LGV), 38 endocervical swabs and 53 vaginal swabs. All specimens were anonimized prior to testing.

### *Conventional microbiological diagnosis*

In order to isolate *N. gonorrhoeae* and *T. vaginalis* from patients suspected of having an STI, endocervical, urethral and vaginal swabs were cultured onto Chocolate agar PolyViteX (bioMèrieux, Marcy-l'Étoile, France), Chocolate agar PolyViteX VCAT3 (bioMérieux), Columbia agar + 5% sheep blood (bioMérieux), Sabouraud dextrose agar (bioMérieux) and Diamond media (Maim SL, Barcelona, Spain). *N. gonorrhoeae* was identified by biochemical methods (Vitek NH cards, bioMérieux), after 48–72 h of incubation in an aerobic atmosphere and *T. vaginalis* was identified by a wet mount examination after 4–5 days of incubation.