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MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

METHODOLOGICAL ARTICLE

EVALUATION OF A MULTIPLEX REAL-TIME PCR ASSAY FOR THE DIAGNOSIS OF SEXUALLY-TRANSMITTED INFECTIONS

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Abstract: Diagnostic methods with simultaneous multi-targeting are applied for the accurate identification of sexually transmitted infections (STIs). The aim of this study was to evaluate a molecular real-time PCR-based assay (Vivalytic STI assay) for the diagnosis of STIs in comparison with other routine methods. Fifteen individuals suspected of having an STI were tested with the Vivalytic STI assay. As a routine testing, 8/15 patients were tested with the Abbott RealTime CT/NG PCR Assay, 5 specimens were tested with the Simplexa HSV 1 and 2 Direct Kit. Two samples were not tested with a routine assay. Clinical specimens were of different origin (rectal, vaginal, pharyngeal, urine, cerebrospinal fluid (CSF)). Ten out of 15 specimens were positive according to routine diagnostic assays. Three were positive for *C. trachomatis* (CT) and 2 for *N. gonorrhoeae* (NG) in single infection, one showed a coinfection with CT and NG. Two samples each were tested positive for Herpes simplex virus 1 (HSV 1) and Herpes simplex virus 2 (HSV 2). All the pathogens detected by routine molecular methods were also detected with Vivalytic STI except for one invalid sample. The Vivalytic STI assay additionally detected *M. hominis* and *U. urealyticum* each in one sample. The assay in the focus of the study showed good agreement with other molecular assays and proved to be a simple and a reliable method for the diagnosis of STIs that could improve the diagnostic methods used at our institution.

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Submitted: June, 2022
Accepted: August, 2022

Key words: sexually transmitted infections, real-time PCR assay, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*

INTRODUCTION

Sexually transmitted infections (STIs) are serious public health concerns that have a profound impact on sexual and reproductive health worldwide.[1]

More than 1 million STIs are acquired every day worldwide.[2] According to the World Health Organization (WHO), there were an estimated 376 million new infections with one of four most common STIs (chlamydia, gonorrhoea, syphilis and trichomoniasis) in 2020.[3] The prevalence of some viral STIs is at a similarly high level, with more than 500 million people infected with the herpes simplex virus (HSV1 or HSV2) and about 291 million women harbouring human papillomavirus (HPV) at any point in time.[4, 5]

The consistent rise in the number of reported STIs in the US and in Europe presents a public health priority requiring an urgent public health response.[6, 7]

The importance of sexually transmitted diseases today is primarily due to their prevalence among the population. However, as a result of untreated or unrecognized infection, they can lead to serious consequences and complications including neurological and cardiovascular disease, infertility, ectopic pregnancy, stillbirths, and increased risk of the Human Immunodeficiency Virus (HIV).[8] Drug resistance for gonorrhoea is also a major threat to controlling these STIs worldwide.[9]

STIs are spread primarily through sexual contact and a minor portion can be transmitted vertically from mother to child during pregnancy, childbirth and breastfeeding.[10]

There are about 30 infection agents, including bacteria, viruses and parasites that are known to be transmitted through sexual contact.[3]

According to the data obtained from the Croatian Institute of Public Health, in the last decade, chlamydia infection makes up the largest share among STIs reports in Croatia (43% in the period 2017-2020). In 2020, there were 12 reports of gonorrhoea, 29 of syphilis, and 119 of chlamydial infection, which is a lower number of infections than in the last five years due to fewer tests and diagnoses during the COVID-19 pandemic.[11]

A large increase in the number of patients and episodes of syphilis during 2020 compared to 2019 was observed in Croatia, so travel and movement restrictions undertaken during the COVID-19 epidemic had no impact on the growing syphilis epidemic among HIV-infected men who have sex with men (MSM) in Croatia.[12]

Reports on chlamydial infection have been on a steady decline over the last decade. Given the numerous asymptomatic and unrecognized cases of sexually transmitted infectious diseases, as well as their underreporting, it is difficult to interpret these data unambiguously. Most people with syphilis and gonorrhoea are MSM, while most people with chlamydial infection are women. In Croatia, no large number or increase in the total number of sexually transmitted diseases is reported, as in most European Union countries, but there is an increase in the proportion of these reports in MSM.[13]

Most STIs are symptomatic; however, asymptomatic carriers are important because they can transmit the infection to other people.[14] Appropriate STI diagnosis and treatment are crucial to prevent transmission, decrease morbidity related to these infections and, overall, to improve health of infected individuals. The accurate identification of asymptomatic and symptomatic STIs depends on the availability of quality diagnostic tests.[15]

For these reasons, sensitive diagnostic methods with simultaneous multi-targeting are applied for diagnosis of STIs.[16, 17]

In this study, we have evaluated the usefulness and performance of the Vivalytic STI test (Bosch Healthcare Solutions, Gerlingen, Germany) for detection of common STI agents using various human samples.

The Vivalytic STI test can directly detect ten different pathogens including two viruses, seven bacteria and one parasite. The following pathogens are detected by the Vivalytic STI Test: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Treponema pallidum*, Herpes simplex virus I, Herpes simplex virus II, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Haemophilus ducreyi*.

MATERIAL AND METHODS

Study design

This was an observational study designed for the clinical evaluation of the Vivalytic STI molecular assay in comparison with other molecular assays used for the routine diagnosis of STIs at the Department of Immunological and Molecular Diagnostics at University Hospital for Infectious Diseases ″Dr. Fran Mihaljević″ (UHID, Croatia).

Study population and clinical samples

Fifteen individuals suspected of having a STI that entered UHID through the Outpatient clinic, the Department for pediatric infectious diseases, the Outpatient clinic for HIV/AIDS and the Diagnostic outpatient unit were included in present study. Clinical specimens included 4 rectal swabs, 3 urines, 3 cerebrospinal fluid (CSF), 2 pharyngeal swabs and one of each - skin, ulcer and vaginal swab.

Routine molecular diagnostic testing

Eight out of 15 patients included in the study were tested using the Abbott RealTime CT/NG PCR Assay (Abbott, Illinois, USA), 5 samples were tested with Simplexa HSV 1 and 2 Direct Kit (Diasorin Molecular LLC, Cypress, USA) according to manufacturer's protocol. Two samples were not tested with a routine assay. After routine molecular diagnosis, residual sample volume was stored at -20 ◦C until used for the evaluation of the Vivalytic STI assay.

Detection of STI agents by Vivalytic Analyser

Urine samples, swabs and CSF can be used for the Vivalytic STI test (Bosch Healthcare Solutions, Gerlingen, Germany) and are processed by the Vivalytic Analyser which is a small, fully automated device platform that is capable of quantitative PCR and uses microfluidics to achieve rapid results. The STI array cartridge contains all reagents needed for testing and does not require sample preparation. The cartridge contains internal controls that also integrate with the Vivalytic Analyzer. The sample input volume is 300 µL. The processing through the device increases the harvest from the sample and multiplies it. As a result, comprehensive results can be obtained even if the sample quantity is very small.

RESULTS

A total of 15 specimens (4 rectal swabs, 3 urines, 3 CSFs, 2 pharyngeal swabs and one of each skin, ulcer and vaginal swab) were tested using the Vivalytic STI asssay, including positive (n=10) and negative (n=3) specimens according to routine diagnostic assays (Simplexa HSV 1 and 2 Direct Kit or Abbott RealTimeTM CT/NG). Among specimens with a positive result using the Abbott RealTime CT/NG (n=6), 3 were positive for *C. trachomatis* and 2 for *N.*

gonorrhoeae in single infection, one showed a coinfection with *C. trachomatis* and *N. gonorrhoeae*. Two samples were tested positive for HSV 1 and two for HSV 2 using the Simplexa HSV 1 and 2 Direct Kit. Two syphilis-suspected samples were only tested with the Vivalytic STI assay (Table 1).

The Vivalytic STI assay showed a concordance of 93.3% (14/15) with the results of other routine diagnostics assays. One sample which tested positive for HSV 1 with the Simplexa HSV 1 and 2 Direct Kit was invalid using the Vivalytic STI test due to invalid control for extraction and amplification during procedure.

The Vivalytic STI assay additionally detected *M. hominis* in a sample tested negative with Abbott RealTime CT/NG PCR Assay and *U. urealyticum* in a sample with *C. trachomatis* and *N. gonorrhoeae* coinfection (Table 1).

Table 1: Performance of the Vivalytic STI assay in comparison with routine methods

Sample	Type of sample	Results of routine testing	Results of Vivalytic STI assay
1	CSF	NA	NEG
2	CSF	NEG	NEG
3	CSF	HSV 1	HSV 1
4	vaginal swab	HSV 2	HSV 2
5	throat swab	NG	NG
6	ulcer swab	NA	NEG
7	urine	CT	CT
8	rectal swab	NEG	*M. hominis*
9	rectal swab	NEG	NEG
10	rectal swab	CT	CT
11	urine	CT	CT
12	rectal swab	CT/NG	CT, NG, *U. urealyticum*
13	urine	NG	NG
14	pharyngeal swab	HSV 1	INVALID
15	skin swab	HSV 2	HSV 2

Legend: NA - not applicable, HSV 1 - Herpes simplex type 1, HSV 2 - Herpes simplex type 2, CSF - cerebrospinal fluid, CT - *Chlamydia trachomatis*, NG - *Neisseria gonorrhoeae*, NEG - negative

DISCUSSION

Sexually transmitted infections cause serious health problems and are associated with significant morbidity and mortality worldwide. Early detection and treatment of STIs is crucial for a better clinical outcome for the patient and lowers the chance of spreading the disease.

The aim of this study was to evaluate a molecular real-time PCR-based assay (Vivalytic STI assay) for the diagnosis of STIs in comparison with other routine methods used at our institution. The Vivalytic STI assay enables the detection of 10 different pathogens from a variety of clinical specimens in a single reaction. It can identify microorganisms that are not detected by diagnostic methods used at laboratories such as ours.

The assay in the study focus showed a good agreement with other molecular assays, although the study population was heterogeneous and the study samples were from different origin (rectal, vaginal, pharyngeal, urine, CSF). All the pathogens detected by routine molecular methods were also detected with Vivalytic STI except for the one invalid sample.

Although the additional detection of *U. urealyticum* and *M. hominis* in two samples comes as a bonus feature to

the results, as we did not have other routine diagnostic methods to detect them with, it must be carefully interpreted in the clinical setting, especially in non-genital samples such as rectal swabs. *U. urealyticum* and *M. hominis* are part of the flora from the genitourinary tract of healthy individuals, so their pathogenic role must be further investigated.

However, identification of these potentially pathogenic microorganisms could be relevant, since first-line therapy may not be adequate for *Ureaplasma* and *Mycoplasma* infections.

Using a platform such as the Vivalytic STI assay that enables multiplex detection could be useful in symptomatic patients as well as in asymptomatic and low prevalence population.

As a point-of-care test (POCT), this assay showed to be an affordable, rapid but, first of all, accurate method for detection of STIs. The easy-to-use platform generates results that are easy to interpret in less than 3 hours.

STI epidemic is a major public health concern and finding the right tool to keep it under control should be a universal goal for all who are affected by it.

The present study showed that the Vivalytic STI assay is a simple and a reliable method for the diagnosis of STIs that improved the diagnostic methods used at our institution.

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