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# Contribution of a molecular test for the diagnosis of genital infection with *Trichomonas vaginalis* and *Mycoplasma genitalium*

## *Contribution d'un test moléculaire pour le diagnostic des infections génitales à* Trichomonas vaginalis *et* Mycoplasma genitalium

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**Abstract.** In the present study, we assessed a recently-marketed molecular test, the S-DiaMGTV™ kit (Diagenode), which provides simultaneous detection of *Mycoplasma genitalium* and *Trichomonas vaginalis* in urogenital samples. Performance characteristics of the S-DiaMGTV™ kit were compared to an in-house PCR for detection of *M. genitalium* and, for first time, with direct observation of genital secretions in wet mounting microscopy for *T. vaginalis*, a routine laboratory method. For *M. genitalium,* out of 66 samples, two negative with the in-house PCR were found positive with the S-DiaMGTV™ kit and two positive with the in-house PCR were found negative with the kit. For *T. vaginalis,* four samples were found positive by the molecular test. Among them, two were previously tested by the wet mounting observation and only one was positive. The kit allows an increase of *T. vaginalis* detection even in a low incidence country. Performances of the kit are in favor of its use in routine laboratory practice.

***Key words:*** *sexually-transmitted infection (STI), Trichomonas vaginalis, trichomoniasis, Mycoplasma genitalium, real time PCR*

**Résumé.** Dans la présente étude, nous avons évalué un test moléculaire récemment commercialisé, le kit S-DiaMGTV ™ (Diagenode), qui permet la détection simultanée de *Mycoplasma genitalium* et *Trichomonas vaginalis* dans des échantillons urogénitaux. Les caractéristiques de performance du kit S-DiaMGTV ™ ont été comparées à celles d'une PCR maison pour la détection de *M. genitalium* et, pour la première fois, à la technique microscopique de l'état frais pour *T. vaginalis*, une méthode de routine utilisée en laboratoire de biologie médicale. Pour *M. genitalium*, sur 66 échantillons, deux négatifs avec la PCR maison ont été trouvés positifs avec le kit S-DiaMGTV ™ et deux positifs avec la PCR maison ont été trouvés négatifs avec le kit. Pour *T. vaginalis,* quatre échantillons ont été trouvés positifs par le test moléculaire. Parmi eux, deux ont été testés par l'observation de montage humide et un seul était positif. Le kit permet une augmentation de la détection de *T. vaginalis* même dans un pays à faible incidence, comme la France. Les performances du kit sont en faveur de son utilisation en pratique courante au laboratoire.

***Mots clés :*** *infection sexuellement transmissible (IST), Trichomonas vaginalis, trichomonose, Mycoplasma genitalium, PCR en temps réel*

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*Mycoplasma genitalium* and *Trichomonas vaginalis* are pathogens responsible for sexually-transmitted infection (STI) [1]. In men, these two pathogens are responsible of non-gonococcal urethritis resulting in urethral discharge and dysuria, but also chronic complications. In women, *M. genitalium* is responsible for cervicitis and pelvic inflammatory disease, whereas *T. vaginalis* causes trichomoniasis, mostly characterized by acute disorders like vaginitis with foamy leucorrhea, intense pruritus and dysuria. These pathogens can also be involved in obstetric chronic complications [2, 3]. However, *M. genitalium* and *T. vaginalis* remain asymptomatic in approximately 70.9% and 61.5% of cases, respectively, regardless of sex [2]. The prevalence of *M. genitalium* infection in the general population worldwide is uncertain, usually between 1 and 3%, while trichomoniasis is likely the most prevalent non-viral STI worldwide [4]. But strong epidemiological discrepancies exist depending on the geographical areas. In France, infection prevalence has been evaluated at 3.4% for *M. genitalium* and 1.7% for *T. vaginalis* in the population of patients undergoing systematic screening for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) [2].

Currently, diagnosis of infections due to *M. genitalium* and *T. vaginalis* can be challenging. For both, culture remains extremely difficult to perform and time-consuming, so it is not routinely used. Consequently, *M. genitalium* is usually detected by gene amplification in routine laboratory practice. Meanwhile, diagnosis of trichomoniasis has been longtime based on direct observation of genital secretions in wet mounting microscopy, and requires a skilled and practiced observer. Unfortunately, this diagnosis approach displays low values of sensitivity that range from 38% to 59% [5, 6]. This lack of performance is mostly due to the high fragility of the parasite, which can make false-negative the detection in cases of low parasite burden and/or extended time between sampling and microscopic analysis. Thus, additional methods have been recently developed in order to circumvent such limitations, *e.g. c*ommercialized nucleic acid amplification tests. Several monoplex real-time PCR assays have been described for the molecular detection of *M. genitalium* [7, 8]. The S-DiaMGTV™ kit, recently developed and distributed by Diagenode (Belgium) is a duplex CE-marked real-time PCR assay validated on urine samples and urogenital swabs. It targets the *mgPa* adhesin and the mg219 genes of *M. genitalium,* and a specific 2 kb repeat sequence of *T. vaginalis* [9-11]. Detection of *M. genitalium* and *T. vaginalis* appears to be a complementary tool in addition to CT/NG detection.

The aim of this study was to assess the performance of the S-DiaMGTV™ assay for routine detection of *M. genitalium* or *T. vaginalis* on urine samples and urogenital swabs, comparing them to our in-house-real-time PCR assay for *M. genitalium* and to direct observation of genital secretions in wet mounting microscopy for *T. vaginalis,* method still used in routine laboratory.

## Materials and methods

### *Samples*

A total of 66 routine samples tested for CT/NG and collected between February 2014 and April 2016 in the Department of bacteriology, University Medical center of Tours (France), were selected according to the results of an in-house TaqMan assay for detection of *M. genitalium* targeting the *mgPa* adhesin gene of *M. genitalium* [12]. These 66 samples were 37 endocervical swabs, 24 male urines, four vaginal swabs and one urethral swab. Results for *C. trachomatis* and *N. gonorrhoeae* have been collected and HIV serology was also collected to complete the STI status of our patients.

### *Diagnostic reference methods*

#### DNA extraction

The nucleic acid extraction had been performed on of clinical specimens by EZ1 instruments (Qiagen, Germany) according to the manufacturer's instructions.

#### In-house PCR assay for detection of *M. genitalium*

Molecular detection of *M. genitalium* was systematically carried out in all the aforementioned samples with the in-house TaqMan PCR assay. Amplification was performed in the Smart Cycler II® thermocycler (Cepheid, United States of America) with the mixture and under the conditions described by Edberg *et al* [12].

#### Wet mounting preparation for detection of *T. vaginalis*

Diagnosis of *T. vaginalis* was routinely achieved by direct microscopic observation of the aforementioned fresh endocervical and vaginal samples by wet mounting preparation. A drop of physiological water, in which swab was discarded, was entirely observed at magnification x400. The positive result was defined as the presence of one or more trichomonads displaying characteristic morphological features ("pear" form, containing axostyle and flagella) and jerky motility [13].

### *S-DiaMGTV™ real-time PCR*

Molecular detection of *M. genitalium* and *T. vaginalis* DNA was systematically carried out in all the aforementioned samples with the CE-marked S-DiaMGTV™ real-time PCR kit (Diagenode, Belgium) according to the manufacturer's instructions on the same frozen DNA extracts used for the in-house PCR assay (*cf* DNA extraction*)*. The 25 µL mixture consisted of 2.5 µL of MGTV primers and hydrol-

**Table 1.** *Mycoplasma genitalium* PCR results for the discrepant extracts.

| Extract n° | Sex | Sample | In-house PCR Result | S-DiaMGTV™ PCR Result |
|---|---|---|---|---|
| 1 | Female | Endocervical swab | Pos (37.55) | Neg |
| 2 | Female | Endocervical swab | Pos (35.64) | Neg |
| 3 | Female | Endocervical swab | Neg | Pos (39.37) |
| 4 | Male | Urines | Neg | Pos (37.9) |

Pos: positive; Neg: negative. Cycle threshold values are indicated in parentheses.

ysis probe each primer, 2.5 µL of primer and hydrolysis probe of DNA exogenous internal control, 5 µL of Diagenode Optima DU Master mix 5x DNA, 10 µL of water for PCR and 5 µL of template DNA. Amplification was performed in the Smart Cycler II® thermocycler (Cepheid, United States of America). The temperature profile consisted of 2 min at 50 °C, 10 min at 95 °C and 20 s at 30 °C, followed by 45 cycles of 15 s at 95 °C, 1 min at 60 °C.

### *Real-time PCR interpretation*

For each PCR run, positive control samples were tested for *M. genitalium* (with an expected cycle threshold Ct value between 33 and 36) and for *T. vaginalis* (with an expected Ct value between 32 and 36). In addition, the negative control sample tested in the same experiment should give no detectable signal before 40 cycles. PCR results were validated only if an accurate amplification of the PCR extraction and inhibition control provided by Diagenode (Belgium) was obtained, with a Ct value between 28 and 30. In accordance with the recommendations of the supplier for the commercial test and the previous published data for the in-house PCR [9, 10]. A real-time PCR was considered to be positive for *M. genitalium* (for in-house and commercial assay) or for *T. vaginalis*, if the cycle threshold (Ct) value of the specific reaction was $< 40$.

### *Data analysis*

The clinical sensitivity and specificity of the commercial test for *M. genitalium* were calculated on the basis of the results obtained from the in-house PCR assay that we used as a proxy for a gold standard for these patients. Because of the absence of systematic direct microscopic observation on male urines and endocervical swabs for the detection of *T. vaginalis* in the laboratory routine, it was not possible to calculate the clinical sensitivity and specificity of the commercial test for *T. vaginalis.*

### *Ethics*

The protocol was submitted to the local ethic committee. No written consent was requested as there was no clinical intervention regarding the healthcare and the study was retrospective.

## Results and discussion

Regarding *M. genitalium*, 62/66 samples were concordant between the in-house PCR and the commercial test, 21 positive samples and 41 negative samples. Two negative samples with the in-house test were found weakly positive with the S-DiaMGTV ™ kit and two weakly positive samples with the in-house test were negative with the commercial kit. Discrepant and reproductible results are presented in *table 1*. The samples concerned, whose results have been checked for both techniques, have a high cycle threshold values indicating a low *M. genitalium* DNA load. These weak *M. genitalium* DNA loads could explain the observed discordances between the two real-time PCR assays. Clinically, in two of these discrepant cases, one positive with the in-house test and one positive with the commercial kit, patients had compatible genital symptoms (urethritis and menorrhagia), which seemed exclude false positives results. For the two other ones, no clinical data were available. The clinical sensitivity of the S-DiaMGTV ™ kit for detection of *M. genitalium* was 91.3% and the specificity was 95.3% (*table 2*).

In all out of the 66 samples, four were found positive for *T. vaginalis* by the PCR kit (*table 3*). Direct microscopic observation was previously performed on two of these four cases (*table 3*). All PCR-negative for endocervical and vaginal samples are also negative for *T. vaginalis* by microscopy. Diagnosis was made in only one case, in a pregnant woman with intra-uterine growth retardation and premature delivery by C-section surgery. This finding confirms a possible role of such infection in the clinical outcome. Indeed, several previous studies showed that women infected with *T. vaginalis* were significantly more exposed to premature delivery preterm, or to give birth to low weight infant [14]. Furthermore, significant association was established between trichomoniasis during pregnancy and mental retardation in children born from infected mothers [15]. Although it was never clearly proven, it underscores how critical it may be to diagnose every case of trichomoniasis during pregnancy in order to treat it and to reduce potential adverse outcomes. Three additional cases of trichomoniasis were discovered thanks to the S-DiaMGTV ™ assay ; one on male urines sample and

**Table 2.** Clinical performance characteristics of the the S-DiaMGTV™ PCR assay for the detection of *Mycoplasma genitalium*.

| | | In-house PCR result | | | |
|---|---|---|---|---|---|
| **Assay** | **Result for *M. genitalium*** | **Positive** | **Negative** | **% sensitivity** | **% specificity** |
| S-DiaMGTV™ kit | Positive | 21 | 2 | 91.3 | - |
| | Negative | 2 | 41 | - | 95.3 |

PPV: positive predictive value; NPV: negative predictive value.

**Table 3.** Patients characteristics for *Trichomonas vaginalis* positive samples screening by PCR (S-DiaMGTV™kit).

| Sample | Sex | Suspected geographic area of contamination | Sample nature | Associated genital pathogens | Wet mounting | Clinical context |
|---|---|---|---|---|---|---|
| 1 | Female | African | Vaginal swab | None | Positive | Premature cesarean delivery intrauterine growth retardation |
| 2 | Male | African | Urines | None | Not done | Urethritis with purulent discharge and pelvic pain |
| 3 | Female | Unknown | Endocervical swab | *Mycoplasma genitalium* | Negative | Menstrual disorders |
| 4 | Female | Unknown | Endocervical swab | *Chlamydia trachomatis* | Not done | Interruption of pregnancy after 8 weeks of amenorrhea |

two on endocervical swabs. This case highlights the fact that trichomoniasis is under-diagnosed in women but also in men. Indeed without a directed request from the clinician, there is no direct microscopic observation made on male urines for the detection of *T. vaginalis*. Such a tool would overcome these limitations and accidentally discovery for a better diagnosis of trichomoniasis regardless of sex.

Regarding cases of trichomoniasis that were detected only by the PCR kit, two were associated with another STI, one with *M. genitalium* infection, and one with *C. trachomatis* endocervicitis. Moreover, on the 21 positive samples for *M. genitalium* by both techniques, 19 are from patients co-infected with *C. trachomatis* or *N. gonorrhoeae*. Among patients tested for HIV, none were seropositive. Although the number of patients in our study is limited, our results support the finding which states that STI co-infections are relatively frequent. In France, 46.2% patients positive for *T. vaginalis* detection were co-infected with *C. trachomatis*, *N. gonorrhoeae* or *M. genitalium,* and 38.6% of *M. genitalium* positive patients were co-infected with *C. trachomatis*, *N. gonorrhoeae* or *T. vaginalis* [2]. Even if it is not recommended to carry out a systematic search for these two pathogens, their role in infections is important especially in certain risk groups (patients exposed to STIs, inmates...). The risk appears particularly high in some populations and ethnicity is an item to consider. In our study, we were able to confirm that two of our four positive cases for *T. vaginalis* by the PCR kit, were from Africa. Our results corroborated those of an American study which revealed higher trichomoniasis prevalence among women of African origin: 13.3% compared to 1.8% among non-Hispanic white women [16].

## Conclusion

The clinical performance of the S-DiaMGTV™ real-time PCR kit established in this study and in previous studies, where the sensitivity and specificity were 100% and 99.9% for *T. vaginalis* [11], 100% and 100% for *M. genitalium*, are in favor of its use in routine laboratory practice for detection of *M. genitalium* and *T. vaginalis.* For trichomoniasis, it is noteworthy that this report is the first one comparing the S-DiaMGTV™ kit with direct observation based on fresh wet mounting. The high sensitivity and specificity are much more adapted to a routine use in comparison with the traditional wet mounting method. Indeed, as previously mentioned, the sensitivity of the wet mounting microscopy is not only unsatisfactory [17], but moreover it decreases drastically with the sampling-analysis delay and the viability of the parasite.

Overall, the S-DiaMGTV™ kit tends to improve the sensitivity of the screening of *T. vaginalis* compared to the wet mounting microscopy. In a context where co-infections of STIs are very frequent, this kit associates the detection

of an emerging pathogen, *M. genitalium*, with that of a preponderant pathogen, *T. vaginalis*. During pregnancy, this kit allows the detection of two pathogens, which are essential to identify in order to prevent potential adverse outcomes. Performances of the test are in favor of its use in routine laboratory practice for detection of *M. genitalium* and *T. vaginalis*, which are actually probably underdiagnosed.

**Conflict of interest:** none of the authors has any conflicts of interest to disclose concerning this article

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