■ BRIEF COMMUNICATION

# Optimization of *Trichomonas vaginalis* Diagnosis during Pregnancy at a University Hospital, Argentina

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**Abstract:** The aim of this study was to evaluate different methods for *Trichomonas vaginalis* diagnosis during pregnancy in order to prevent maternal and perinatal complications. A total of 386 vaginal exudates from pregnant women were analyzed. *T. vaginalis* was investigated by 3 types of microscopic examinations direct wet mount with physiologic saline solution, prolonged May-Grunwald Giemsa (MGG) staining, and wet mount with sodium-acetate-formalin (SAF)/methylene blue method. PCR for 18S rRNA gene as well as culture in liquid medium were performed. The sensitivity and specificity of the microscopic examinations were evaluated considering the culture media positivity or the PCR techniques as gold standard. The frequency of *T. vaginalis* infection was 6.2% by culture and/or PCR, 5.2% by PCR, 4.7% by culture, 3.1% by SAF/methylene blue method and 2.8% by direct wet smear and prolonged MGG staining. The sensitivities were 83.3%, 75.0%, 50.0%, and 45.8% for PCR, culture, SAF/methylene blue method, and direct wet smear-prolonged MGG staining, respectively. The specificity was 100% for all the assessed methods. Microscopic examinations showed low sensitivity, mainly in asymptomatic pregnant patients. It is necessary to improve the detection of *T. vaginalis* using combined methods providing higher sensitivity, such as culture and PCR, mainly in asymptomatic pregnant patients, in order to prevent maternal and perinatal complications.

Key words: *Trichomonas vaginalis*, diagnosis, pregnancy, Argentina

Trichomoniasis is a sexually transmitted infection associated with urethritis, vaginitis, cervicitis, pelvic inflammatory disease, and tubal infertility [1]. Besides, trichomoniasis promotes the transmission and infectivity of HIV [2]. During pregnancy, hormonal changes predispose to a higher incidence of lower genital tract infections, which can lead to maternal and perinatal complications; consequently, *Trichomonas vaginalis* diagnosis is of great relevance as such infections are related to premature rupture of membranes, preterm delivery, and low birth weight [3]. Whereas 50% of infected women will develop clinical symptoms during the next 6 months, up to 10-50% of them remain asymptomatic [4]. Despite being adequately treated, women usually become reinfected by sexual contact with their untreated partners, as men are usually asymptomatic carriers [5].

Microscopic examination is traditionally performed for *T. vaginalis* diagnosis as it is quick and inexpensive, but depends on the microscopists' skill and on the prompt transport and processing of the sample, so as to avoid the loss of parasite motility [1]. Also, it has limited sensitivity mainly in asymptomatic patients [1]. That is the reason why it is necessary to carry out more sensitive methodologies [1,6]. Therefore, culture in liquid medium is regarded as the most accurate method (gold standard) for the diagnosis of trichomoniasis [7-10]. Also several authors reported the nucleic acid amplification tests (NAATs) as more sensitive methods [6-9]. The aim of this work was to optimize and evaluate different methods for diagnosis of *T. vaginalis* during pregnancy in order to prevent maternal and perinatal complications.

Total 386 vaginal exudates from pregnant women were surveyed from 1 April 2010 to 31 August 2011. All patients were examined at the Obstetrics Clinic at the Hospital de Clínicas of the University of Buenos Aires, and were subjected to clinical and colposcopic evaluations. These evaluations allowed the

classification of the patients into asymptomatic and symptomatic. This study was performed under Hospital Ethics Committee's approval. All women included in this study gave their written informed consent to participate.

Vaginal discharge samples were extracted from the vaginal fornix. The microbiologic study of vaginal discharge samples for the diagnosis of trichomoniasis included microscopic wet smear examination with 1 ml of physiologic saline solution, as well as Gram and prolonged May-Grunwald Giemsa (MGG) stained smears. Another examination included a microscopic wet smear examination with sodium acetate-acetic acid-formalin (SAF)/methylene blue (methylene blue 0.5 ml and SAF 0.5 ml) [11]. Also, culture in liquid medium (modified thyoglicolate medium) for *T. vaginalis*, with a 7-day incubation period at 37°C in an atmosphere of 5% $CO_2$ [10,12]. Additional tests were performed as vaginal discharge pH obtained using indicators strips (range 3-7), and fishy-odor test with 1 ml of 10% KOH with subsequent microscopic wet smear.

*T. vaginalis* culture was performed at bedside and then incubated at proper conditions not more than 1 hr after collection. Microscopic examinations were also carried out within less than 1 hr following sample collection. Leukocyte quantification was assessed by May-Grunwald Giemsa staining and was expressed as leukocyte numerical range per field (400x) calculating the average leukocyte count within 10 non-adjacent microscopic fields.

Also, the search for *T. vaginalis* was carried out by detection of a specific region of the 18S rRNA gene of *T. vaginalis* as described by Mayta et al. [13]. The analytical sensitivity for this PCR method was assessed in our laboratory conditions from a culture of $3.2 \times 10^3$ parasites to which DNA was extracted and serial 10-fold dilutions were made. Concentrations of Taq, magnesium, and primers were optimized in the PCR reaction

to obtain a sensitivity of less than 1 parasite per reaction tube (final sensitivity: 0.5 parasites per reaction tube, 50-100 parasites/ml in the original sample). For each clinical sample, a PCR for the human β-globin gene was carried out as a control for the presence of amplifiable DNA and PCR inhibitors [13]. If any sample resulted in β-globin negative, it was not included in this study.

The different methods employed were compared considering culture or PCR positivity as true positive values (expanded gold standard). Both sensitivity and specificity were estimated with a 95% confidence interval (CI) (EPI INFO 6.0). Odds ratio (OR) was estimated to relate vaginal inflammatory status with symptomatology.

Infection by *T .vaginalis* was diagnosed in 24 out of 386 pregnant patients (6.2%) (95% CI: 4.1-9.2%) using an expanded gold standard (positive culture and/or PCR). Fourteen samples were positive by both methodologies, 6 were positive only by PCR (5.2%) (95% CI: 3.3-8.0%) and 4 only by culture (4.7%) (95% CI: 2.9-7.4%). The presence of *T. vaginalis* was also detected by wet mount with SAF/methylene blue in 12 positive samples (3.1%) (95% CI: 1.7-5.5%) but in 11 samples (2.8%) (95% CI: 1.5-5.2%) using prolonged MGG staining and direct wet mount with physiologic saline solution. Table 1 shows the sensitivity, specificity, positive predictive value, negative predictive value, and area under the ROC curve of each diagnostic method for *T. vaginalis* in the total pregnant patients. The sensitivities were 75.0% (95% CI: 34.9-96.8%), 100% (95% CI: 63.1-100%), 100% (95% CI: 63.1-100%), and 87.5% (95% CI: 47.4-99.7%) in symptomatic patients and 87.5% (95% CI: 61.7-98.5%), 62.5% (95% CI: 35.4-84.8%), 25.0% (95% CI: 7.3-52.4%), and 25.0% (95% CI: 7.3-52.4%) in asymptomatic patients, for PCR, culture, SAF/methylene blue method, and direct wet smear with prolonged MGG

**Table 1.** Sensitivity, specificity, positive predictive value, negative predictive value, and area under the ROC curve of the different diagnostic methods for *T. vaginalis* in pregnant patients

| Method | Sensitivity | | Specificity | | Positive predictive value | | Negative predictive value | | Area under curve ROC | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | (95% CI) | % | (95% CI) | % | (95% CI) | % | (95% CI) | % | (95% CI) |
| Wet mount with saline[a] and prolonged Giemsa[b] | 45.8 | (26.2-66.8) | 100 | (99.0-100) | 100 | (71.5-100) | 96.5 | (94.0-98.1) | 0.73±0.07 | (0.60-0.86) |
| Wet mount with SAF/ methylene blue | 50.0 | (29.7-70.4) | 100 | (99.0-100) | 100 | (73.5-100) | 96.8 | (94.3-98.3) | 0.75±0.07 | (0.62-0.88) |
| Culture | 75.0 | (53.3-90.2) | 100 | (99.0-100) | 100 | (81.5-100) | 98.4 | (96.5-99.4) | 0.88±0.06 | (0.77-0.98) |
| PCR | 83.3 | (62.6-95.3) | 100 | (99.0-100) | 100 | (83.2-100) | 98.9 | (97.2-99.7) | 0.92±0.05 | (0.82-1.00) |

ROC, receiver operating characteristic; CI, confidence interval; SAF, sodium acetate-acetic acid-formalin.
[a]physiological saline solution.
[b]May-Grunwald Giemsa.

staining, respectively. The specificity was 100% (95% CI: 99.0-100%) for all the assessed methods in both symptomatic and asymptomatic patients. Twenty-five percent of the samples were β-globin negative.

Of the 24 patients with trichomoniasis, 16 were asymptomatic and their colposcopic examinations were normal. The microscopic examinations were negative for *T. vaginalis* in 12 out of 16 of these patients. On the other hand, 8 out of 24 patients were symptomatic with cervicocolpitis, and for these 8 patients at least 1 out of the total microscopic examinations for *T. vaginalis* was positive. A vaginal inflammatory status with a leukocyte numeric range >10 per field (400x) was significantly associated to symptomatology, with OR 45 00 (2.55<OR<2,198. 34) (*P*<0.003).

In the present study, which included both symptomatic and asymptomatic pregnant women, a frequency of 6.2% of *T. vaginalis* infection was observed by culture and/or PCR. The prevalence values obtained during pregnancy differ in various studies regarding the populations examined. Madico et al. [14] described prevalence of *T. vaginalis* of 6.6% by culture media and of 9.4% by culture plus PCR in symptomatic and asymptomatic patients. In addition, we have previously reported a prevalence of 4.0% by culture in symptomatic and asymptomatic pregnant women [12]. Nevertheless, Gondo et al. [15] registered much lower prevalence of *T. vaginalis* by culture (0.4%) in symptomatic and asymptomatic pregnant women. To date, no prevalence data of *T. vaginalis* by culture and PCR have been published in Argentina.

On the other hand, *T. vaginalis* prevalence differs according to the methodology employed. In this study, prevalences obtained by microscopic examinations were lower than those observed by culture and PCR. Likewise, in the literature, lower values are observed by microscopic examinations than by culture and PCR, although variations were observed considering the population analyzed. In Brazil, Simoes et al. [16] reported prevalences similar to our results, 2.1% for microscopic examinations in pregnant women with and without symptoms. Moreover, we have previously described higher prevalences of *T. vaginalis* (4.5%) for microscopic examinations in both symptomatic and asymptomatic pregnant women [17]. Di Bartolomeo et al. [18], in Argentina, also showed higher prevalences to the ones found in our present study (3.5%) for wet mounts with physiological saline solution and prolonged MGG staining, although patients were symptomatic pregnant women.

As reported by several studies, the sensitivity of the microscopic examination ranges between 35% and 80% [1,4]. In this study, the sensitivity of the different microscopic examinations was low (45.8-50.0% according to the staining as shown in Table 1). The sensitivity decreases to 25% in all microscopic examination techniques in the group of asymptomatic patients. Radonjic et al. [19] reported a sensitivity of 66.7% for wet mount and a sensitivity of 52.4% for prolonged MGG staining, in patients with gynecological complications. Likewise, Costamagna [11] informed a sensitivity of 58.3% for the wet mount with physiologic saline solution, and also described a higher efficiency of the wet mount with SAF/methylene blue method. The low sensitivity of the microscopic examinations in asymptomatic patients may be probably due to the lower load of parasites in these patients. Although the sensitivity was low, the specificity of the different microscopic examinations was high (100%), as previously described [19].

Culture in liquid medium (modified thyoglicolate) as a unique method detected 75% of the genital infections by *T. vaginalis*. Using PCR, the detection increased to 83% of the infected women. Randongic et al. [19] reported similar results with a sensitivity and a specificity of PCR of 81.0% and 97.2%, respectively. Likewise, Madico et al. [14] described a sensitivity of culture and PCR of 70% and 97%, respectively. Using a set of primers targeting a conserved region of the β-tubulin genes of *T. vaginalis*, they also described a specificity of 100% of culture and of 97.8% by PCR. In addition, Paul et al. [20] observed a significant agreement between the PCR and culture with a set of primers which amplifies a 102 bp genomic sequence of adhesine gene. Also, Shipitsyna et al. [21] reported sensitivities of the PCRs of 94.3-100% and a specificity of 100% using 5 PCR assays and considering the real-time multiplex PCR as the reference assay. It should be mentioned that there is no standardized PCR method for *T. vaginalis* diagnosis.

The search for *T. vaginalis* by means of the liquid culture medium requires a thorough daily microscopic observation during 7 days. In those patients with direct negative microscopic examinations, growth of the parasite in culture was observed between the 2nd and the 7th day.

The 4 samples detected only by culture and negative by PCR, after the dilution of the original sample, were found positive. In 2 of these samples, it is presumed that an excess of genetic material could have negatively interfered with the PCR, as Mayta et al. [13] also pointed out when first described the PCR we used in this study. They posed that because of the back-

ground of the samples they could have a positive result after a first negative result for *T. vaginalis* DNA amplification, once the sample was diluted. In the other 2 samples positive by culture but negative by PCR, the presence of inhibitors is presumed, because a negative result was obtained for the amplification of the β-globin gene. However, as we first stated, β-globin negative samples were not included for the sensitivity and specificity analyses in comparison of the different techniques.

As 25% of the samples were β-globin negative, it is presumed that the presence of inhibitors might also inhibit the detection of *T. vaginalis* by PCR yielding false negative results with both negative cultures and microscopic examinations. For this reason, it might be inferred that the frequency of infection by *T. vaginalis* might be even higher.

It is worth mentioning that in most of the asymptomatic pregnant women, *T. vaginalis* diagnosis was only performed by culture and/or PCR, because the microscopic examinations were mostly negative, while the presence of this parasite was mainly associated with *Lactobacillus* spp., pH was ≤4.5 and negative fishy-odor test.

The results of this study indicated that, while there is not a standardized protocol for the detection of *T. vaginalis* infection, it should be necessary to improve it by using combined methods with higher sensitivity and area under curve ROC, such as culture and PCR, mainly in asymptomatic pregnant patients, to increase the power of detection in order to implement an early and adequate treatment, and thus, prevent possible maternal and perinatal complications.

## ACKNOWLEDGMENTS

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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