Modified Field’s staining—a rapid stain for *Trichomonas vaginalis*

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Abstract

*Trichomonas vaginalis*, a flagellate protozoan parasite commonly found in the human genitourinary tract, is transmitted primarily by sexual intercourse. Diagnosis is usually by in vitro culture method and staining with Giemsa stain. There are laboratories that use Gram staining as well. We compared the use of modified Field’s (MF), Giemsa, and Gram stains on 2 axenic and xenic isolates of *T. vaginalis*, respectively. Three smears from every sediment of spun cultures of all 4 isolates were stained, respectively, with each of the stains. We showed that MF staining, apart from being a rapid stain (20 s), confers sharper staining contrast, which differentiates the nucleus and the cytoplasm of the organism when compared to Giemsa and Gram staining especially on parasites from spiked urine samples. The alternative staining procedure offers in a diagnostic setting a rapid stain that can easily visualize the parasite with sharp contrasting characteristics between organelles especially the nucleus and cytoplasm. Vacuoles are more clearly visible in parasites stained with MF than when stained with Giemsa.

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Keywords: *Trichomonas vaginalis*; Giemsa stain; Gram stain; Modified Field’s stain; Rapid

1. Introduction

Trichomoniasis, one of the most important infectious diseases, occurs worldwide, in both urban and rural country (Rein and Zenilman, 2000). It has been estimated that more than 170 million people worldwide are infected with *Trichomonas vaginalis* per year (WHO, 1995). Diagnosis is usually by in vitro culture technique (Schee et al., 1999; Schwebke and Lawing, 2002; Stary et al., 2002) and staining with either Giemsa (McMillan, 2002; Radonjic et al., 2006) or Gram stain (Barberis et al., 2002; Harrington and Williams, 1999). A more rapid staining technique could facilitate identification especially made from vaginal swabs. The present study attempts to use modified Field’s (MF) stain, previously used in malarial staining, and compare its characteristics with Giemsa and Gram staining for the purposes of seeking alternative rapid staining protocol for *T. vaginalis*.

2. Materials and methods

2.1. Sample collection of *T. vaginalis*

2.1.1. Axenic isolates

Two axenic isolates of *T. vaginalis* were obtained from the Department of Parasitology, University of Queensland, Brisbane, Australia, and continuously maintained in Diamond TYM medium and subcultured once every 4 days.

2.1.2. Xenic isolates and urine samples

Vaginal swabs and urine samples were collected, respectively, from 80 female patients seen at the Obstetric and Gynecology clinic in a local hospital complaining of vaginal discharges and itching. The vaginal swabs was subsequently inserted into culture tubes containing 3 mL of Hollander medium (Hollander, 1976) supplemented with 10% heat-inactivated horse serum (Gibco Laboratories, Life Technologies). Two positive cultures for *T. vaginalis* were continuously maintained in Hollander medium supplemented with 10% heat-inactivated horse serum and subcultured once every 3 days. Urine samples were obtained from the patients providing vaginal swabs, which was subsequently centrifuged (2000 × g, 10 min) within 1 h of collection.
2.2. Smears

Sediment from both axenic and xenic cultures was used to make thin smears on clean glass slides for staining. Sediment from xenic cultures was also inserted into freshly collected urine samples and subsequently centrifuged (2000 × g, 5 min). Smears were made from spiked urine samples for staining purposes. The smears from every sediment at the bottom of culture tubes were performed in triplicates. The slides were examined by 3 others who independently read the unlabeled slides blindly.

2.3. Giemsa staining method for T. vaginalis

Smeared slides containing T. vaginalis were dried and fixed with methanol for 30 s and stained with Giemsa stain (Sivanandam and Mak, 1974) and allowed to air dry at room temperature for 15 to 20 min. The dried slides were subsequently rinsed with distilled water and allowed to dry at room temperature and mounted using DPX. The slides were observed under 400× magnification (Table 1).

2.4. Gram staining method of T. vaginalis

Smears of sediment containing T. vaginalis were dried in room temperature for 15 to 20 min and stained using Gram stain (Stefanski et al., 2010) following a standardized 4-step staining protocol with Hucker’s modification and Becton Dickinson (Becton Dickinson and Company) Gram stain kits.

2.5. MF staining method for T. vaginalis

Parasites were stained with the MF stain according to the method by Sivanandam and Mak (1975). Smears of sediment containing T. vaginalis were dried at room temperature for 15 to 20 min, and then, subsequently, 8 drops of Field’s stain B (0.2% solution of eosin in methanol) were added followed by 16 drops of Field’s stain A onto the smear. Slides were then slightly agitated for 15 s before being rinsed for 2 s under a brisk stream of water, dried at room temperature, and mounted using DPX. The slides were then observed under 400× magnification.

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Table 1: Comparison of 3 different staining methods for identification of T. vaginalis:

<table>
<thead>
<tr>
<th>Factor</th>
<th>Giemsa stain</th>
<th>MF stain</th>
<th>Gram stain</th>
</tr>
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<tbody>
<tr>
<td>Staining time</td>
<td>Approximately 20 min</td>
<td>Approximately 20 s</td>
<td>Approximately 5 min</td>
</tr>
<tr>
<td>Staining reagents</td>
<td>3 types of reagents:</td>
<td>2 types of reagents, Field stain power</td>
<td>4 types of reagents:</td>
</tr>
<tr>
<td></td>
<td>(1) 3.8-g Giemsa stain (Revector brand, Hopkin and Williams brand, or Gurr’s R66 brand);</td>
<td>(1) crystal violet;</td>
<td>(1) 6.25-g Field’s stain A;</td>
</tr>
<tr>
<td></td>
<td>(2) 500-mL pure methanol (AnalaR BDH UK brand);</td>
<td>(2) iodine acetone (50:50 v/v);</td>
<td>(3) 500-mL glycine (AnalaR BDH UK brand)</td>
</tr>
<tr>
<td>Technique for preparation</td>
<td>Mix 3 types of reagents in mortar ↓</td>
<td>Field’s stain A in 500 mL of hot water ↓</td>
<td>Readymade</td>
</tr>
<tr>
<td></td>
<td>Pour mix reagents into a clean bottle ↓</td>
<td>Shake thoroughly ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Keep at 37 °C incubator for minimum 24 h ↓</td>
<td>After mixing properly, filter with the filter paper and pour into a clean bottle ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shake 5–6 times occasionally ↓</td>
<td>Shake thoroughly ↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After 24 h, filter with the filter paper ↓</td>
<td>After mixing properly, filter with the filter paper and pour into a clean bottle ↓</td>
<td></td>
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<tr>
<td></td>
<td>Ready to use ↓</td>
<td>Ready to use ↓</td>
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| Time to generate the stain | Tedious; the stain must be kept at minimum of 24 h in the incubator before it can be used |
| Shelf lives | 1–2 years |
| Staining performance on T. vaginalis | The stain does provide contrast for morphologic identification of T. vaginalis, though the contrast was lesser on parasites from spiked urine samples |

Simple, fast, preparation; time less than 30 min. The stain can be used immediately 1–2 years. The stain provided high contrast for morphologic identification of T. vaginalis compared to other methods.

Simple and very fast 1 year The stain performance is the poorest and no contrast for morphologic identification of T. vaginalis compared to other methods.
3. Results and discussion

The staining characteristics of all 3 slides from the same sediment, respectively, showed consistency when stained with the respective stain. *T. vaginalis* obtained from vaginal swabs or urine samples would contain bacteria, and therefore, the present study compared the use of MF stain on xenic isolates as well as *T. vaginalis* spiked urine samples to assess if staining characteristics could be influenced by the bacteria and the contents of urine.

Giemsa stained nucleus and cytoplasm dark and light purple, respectively. However, the flagella of *T. vaginalis* were difficult to visualize (Fig. 1A). MF stained the nucleus, cytoplasm, and flagella of *T. vaginalis* reddish purple, light purple, and dark reddish purple, respectively, with lesser background staining when compared to Giemsa (Fig. 1C). Vacuoles, when stained with MF, are more clearly visible in parasites from xenic isolates when compared to Giemsa staining.

Samples from spiked urine culture tubes showed that the contrast was sharper for diagnosis using MF (Fig. 2C) and Giemsa (Fig. 2A) than Gram (Fig. 2B) staining. Giemsa and MF can be obtained commercially in powder form, whereas Gram stain is usually commercially available in solution form. Powder forms have longer shelf-life.

Giemsa stain (McMillan, 2002; Radonjic et al., 2006) or Leishman’s stain have been used previously for the detection of trichomoniasis as it was found to be simple, reliable, and inexpensive.

MF stain has been used previously for the detection of malaria (Fenton and Innes, 1945; Field, 1940; Reilly et al., 1997), filaria (Sivanandam and Mak, 1975), leishmaniasis (Chunge et al., 1989), *Acanthamoeba* spp. (Pirehma et al., 1999), and trypanosomes (Sindato et al., 2007). Pirehma et al. (1999), which concurs with our finding that MF is easy to prepare, and its staining protocol only lasts 20 s and provides a very good contrast when compared to Wright’s stain, Giemsa stain, Ziehl–Neelsen stain, and trichrome stain.

Gram stain is a widely used method for identification of some bacteria such as *Neisseria gonorrhoeae*, *Chlamydta trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum* (Barberis et al., 2002), fungi (Barberis et al., 2002; Brown and Wu, 1986), *Pneumocystis carinii* (Brown and Wu, 1986), micorosporidia (Cali et al., 1991), and *T. vaginalis* (Barberis et al., 2002; Harrington and Williams, 1999).

Harrington and Williams (1999), however, did report that Gram-stained *T. vaginalis* require skilled expertise and confidence for detection. This observation of theirs concurred with our finding in that the staining characteristics between the nucleus and cytoplasm showed poor contrast. Although staining and in vitro culture techniques are frequently used detection methods for *T. vaginalis* in laboratories, diverse molecularly based diagnostic methods, such as hybridization assay and polymerase chain reaction,
have been used to detect T. vaginalis but vary widely in their sensitivities and specificities for detection of T. vaginalis (Rojas et al., 2004). However, cost and time is still a consideration for many laboratories especially from the developing world.

The Field’s staining method (Field et al., 1963) was used in this study following the protocol of Sivanandam and Mak (1974) with a slight modification of timing where the original 10 to 12 s for malarial staining was extended to 15 to 20 s in the present study to optimize staining results for detecting T. vaginalis. Castillo and Rojas (1997) reported that MF stain was more sensitive than Giemsa (78.7% versus 70.5%), the diagnosis of American cutaneous leishmaniasis.

In the present study, the results suggest that both Giemsa and MF stain are equally good for detecting T. vaginalis. The staining procedure for MF stain required only 20 s in the present study as opposed to 20 min with Giemsa staining. Furthermore, it would take 24 hrs for Giemsa to completely dissolve prior to use. However, MF stain has several advantages compared to Giemsa stain such as the ease and rapidity in preparation, making it suitable for both laboratory and field use.

In conclusion, we establish for the first time a rapid staining method using MF stain to facilitate the identification of T. vaginalis, as not only the protocol is simple and rapid but also the contrast showing nucleus, cytoplasm, vacuoles, and flagella was clearly visible and is equal if not better than Giemsa. MF staining offers a rapid staining protocol in laboratories and is recommended that it be used widely. The only observation to take note is that staining must be done on slides within 12 h after smearing as the contrast becomes lesser when the duration of fixing and staining increases more than 12 h. We also did not observe any difference in staining characteristics on parasites from axenic and xenic isolates, respectively.

Acknowledgment

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References

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