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### Diagnosis of trichomoniasis in pap smears; How effective is it?

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#### ABSTRACT

*Trichomonas vaginalis* is sometimes seen in Pap smears where it is reported, but because emphasis is placed on malignant cells in Pap smears, not much is done to search for this parasite in smears. In this experiment, cervical and vaginal specimens were examined microscopically in wet preparations and simultaneously by the conventional Papanicolaou method for the presence of *Trichomonas vaginalis*. The specimens were also cultured for the presence of *Trichomonas vaginalis* and PCR done to confirm the presence of the organisms. When compared with positive results of culture and PCR, wet preparations had the highest sensitivity of 81.58% followed by 65.77% of diagnosis based on both perinuclear halo and *T. vaginalis*. Presumptive diagnosis based on perinuclear halo alone was 52.63% while diagnosis based on identification of organisms in Pap smear was 42.11%. Therefore, the effectiveness of diagnosis of *T. vaginalis* in Pap smears is about 65.77% and it should not be used to exclude trichomoniasis.

**Key words:** Papanicolaou smears, *Trichomonas vaginalis*, microscopy,

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#### INTRODUCTION

*Trichomonas vaginalis* is a flagellated protozoan which causes Trichomoniasis and is the most common curable sexually transmitted disease worldwide [1,2]. Apart from human papillomavirus, trichomoniasis is the most common sexually transmitted infection in the United States today. Among both women and men, the association of *T. vaginalis* with human immunodeficiency acquisition and transmission has been shown in several studies [1]. In women, trichomoniasis may play a role in development of cervical neoplasia, postoperative

infections, and adverse pregnancy outcomes and as a factor in atypical pelvic inflammatory disease and infertility. In men, trichomoniasis has emerged as a cause of nongonococcal urethritis and as contributing to male factor infertility [2]. The most common method of diagnosis is via overnight culture [3,4] with a sensitivity range of 75-95% [5]. Methods, such as rapid antigen testing and transcription-mediated amplification, have also been used and are said to have greater sensitivity, but are not in widespread use [5]. The presence of *T. vaginalis* can also be diagnosed by PCR, using primers specific for GENBANK/L23861 [6]. The Pap smear is a routine screening test used for the detection of cervical abnormalities and precancerous dysplastic changes of the uterine cervix [7]. It also detects certain viral, bacterial, and fungal infections of the cervix and vagina [8]. There is also epidemiological and experimental evidence that Pap smears are beneficial in detecting infections that are risk factors associated with cervical cancer, such as human papilloma virus [9]. The aim of this study was to determine the suitability of Pap smear in the detection of *Trichomonas vaginalis* in cervical and vaginal specimens.

## MATERIALS AND METHODS

Three hundred cervical and vaginal specimens in cotton wool tipped applicators which were sent for microscopy were simultaneously examined by the conventional Papanicolaou method, culture and by PCR for the presence of *Trichomonas vaginalis* in some hospitals in Southern and Western Nigeria.

### Papanicolaou method

Each specimen was smeared on a clean grease free slide and fixed in ether-alcohol for 30 minutes. The specimens were then stained by the Papanicolaou method as follows: Harris's haematoxylin without acetic acid for 5 minutes, rinsed in tap water and differentiated in 1% acid-alcohol for 30 seconds and blued in Scott's water for 2 minutes. Smears were taken to 95% alcohol and stained in OG6 for 2 minutes, rinsed in 95% alcohol and stained in EA 35 for 2 minutes. Smears were then taken to two changes of absolute alcohol, xylene and mounted in DPX. The stained smears were examined under the light microscope at low and high power objectives for the presence of *Trichomonas vaginalis* and perinuclear halo.

### Examination of wet preparation

Each cotton wool tipped applicator was subsequently rinsed in a test tube containing about 2 ml normal saline. The content was poured onto a clean glass slide and examined under the light microscope for the presence of a rapidly moving organisms.

### Culture of *T. vaginalis*

#### Preparation of culture medium and culture

Kupferberg *Trichomonas* medium was prepared by dissolving 23.5g of the Kupferberg *Trichomonas* base (QUELAB, Canada) in 950 ml of distilled water with the aid of heat, sterilized in an autoclave for 15 min at 15 lb pressure (121°C) and cooled. 50ml of heat inactivated (55-60°C) bovine serum was added. Antibiotics (penicillin G and streptomycin) and antifungal (Amphotericin B) were added to the mixture and stored at 4°C. About 15ml of the medium was put in a culture tube and heated to 37°C for 15 min. The cervical and vaginal swabs were placed into the medium and incubated at 37°C for 7 days after which they were examined

microscopically. The medium was washed two times in sterile phosphate buffered saline (PBS) pH7.2 and subjected to DNA extraction.

### DNA extraction, primers and PCR

The cultures were washed twice in sterile phosphate buffered saline at pH7.2 and suspended in 400µl T/E buffer. DNA extraction was performed using SDS and proteinase K followed by CTAB/NaCl. The presence of DNA was confirmed by electrophoresis prior to PCR amplification. Primers based on *T. vaginalis* DNA were used to amplify a 300 bp piece of genome (TIB MOLBIOL, Germany) while the PCR reaction was performed using the automated thermal cycler (Eppendorf master cycler gradient).

## RESULTS

**Table 1 Pap smear, wet preparation and PCR**

Total number of specimens examined	300	%
Total positive for perinuclear halo (suggestive of <i>T. vaginalis</i> )	20	6.67
Total organisms seen in Pap smears	16	5.33
Total positive for perinuclear halo (suggestive of <i>T. vaginalis</i> ) and <i>T. vaginalis</i>	25	8.33
Total positive during wet preparation for microscopy	31	10.33
Total positive by culture and PCR	38	12.67

Of the 300 specimens examined by the Pap technique, 24 (8%) had perinuclear halo suggestive of *T. vaginalis* while the organisms were seen in 15 (5%) of them. 30 (10%) of the specimens had both *T. vaginalis* and perinuclear halo. In wet preparations under the light microscope, 36 (12%) of the specimens had *T. vaginalis*.

**Table 2 Percentage sensitivity in comparison with culture and PCR**

Investigations	No	Sensitivity %
Total number of specimens positive by culture and PCR	38	100
Total positive for perinuclear halo (suggestive of <i>T. vaginalis</i> ) in Pap smear	20	52.63
Total organisms seen in Pap smears	16	42.11
Total positive for perinuclear halo (suggestive of <i>T. vaginalis</i> ) and <i>T. vaginalis</i>	25	65.77
Total positive during wet preparation for microscopy	31	81.58

When compared with positive results of culture and PCR, wet preparations had the highest sensitivity of 81.58% followed by 65.77% of diagnosis based on perinuclear halo and *T. vaginalis*. Presumptive diagnosis based on perinuclear halo alone was 52.63% while diagnosis based on identification of organisms in Pap smear was 42.11%

## DISCUSSION

Papanicolaou is the best staining method in cytology, because it helps to effectively differentiate malignant cells from non-malignant cells. It also stains the cytoplasm and its contents [10]. Its ability to differentiate acidophilic materials from basophilic materials as well as its ability to stain non-cellular substances such as fibrin, crystals and pigments, makes it an essential stain in

cytology [10]. *T. vaginalis*, the causative organism for trichomoniasis is the most common curable sexually transmitted organism worldwide [1,2]. It parasitizes both males and females where it is sometimes asymptomatic in the early stages of the infection. *T. vaginalis* infection is said to play a role in the development of cervical neoplasia, postoperative infections, and adverse pregnancy outcomes and as a factor in atypical pelvic inflammatory disease and infertility [2]. There is also epidemiological and experimental evidence that Pap smears are beneficial in detecting infections that are risk factors associated with cervical cancer, such as human papilloma virus [9]. Several methods of diagnosis of trichomoniasis exist. There is the easiest method which involves examination of a wet preparation under the microscope where the organisms are seen moving rapidly in all directions. Other methods include overnight culture [11,3,4,5], rapid antigen testing, and transcription-mediated amplification [5] and by PCR [6]. Pap smear is a routine screening test used for the detection of cervical abnormalities and precancerous dysplastic changes of the uterine cervix [7]. It also detects certain viral, bacterial, and fungal infections of the cervix and vagina [8]. In this experiment, wet preparations of cervical and vaginal smears and Papanicolaou stained smears were examined and compared with results obtained from PCR after 7 days culture. The presence of perinuclear halo in the epithelial cells was used as a presumptive diagnosis for *T. vaginalis*. Pap smears are not superior to wet preparations in the detection of *T. vaginalis* as shown in tables 1 and 2. Culture is a very sensitive method of detecting *T. vaginalis* but it is expensive and time consuming. It is concluded that while *T. vaginalis* should be reported in cervical and vaginal Pap smears, its absence in these smears is not an indication for absolute absence of the organism in the patient.

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