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The sensitivity, specificity and accuracy of liquid based cytology (LBC) in the diagnosis of vaginal candidiasis in Harare, Zimbabwe

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Abstract
Background: The primary role of Liquid Based Cytology (LBC) is the detection of atypical epithelial cells of the cervix; however, it can play a marginal role in the diagnosis of infections of the vagina. A few studies have evaluated the performance of a conventional Pap smear for the diagnosis of Candidiasis. However, there is no published data on the performance of Liquid Based Cytology (LBC) (a superior sample preparation method) for the detection of vaginal *Candida*.
Objective: To determine the sensitivity, specificity, NPV, PPV, and accuracy of LBC in the diagnosis of vaginal Candidiasis.
Design: Cross sectional descriptive study**.**
Setting: Cimas Medical Laboratories.
Subjects: Women presenting with vaginal discharge**.**
Materials and Methods: Fixed LBC slides were stained using the Papanicolaou stain and evaluated for the presence of *Candida.* A High Vaginal Swab (HVS) culture on Sabouraud Dextrose Agar (SDA) culture was used as the gold standard. A Cohen's Kappa test was done to determine the level of agreement between LBC and HVS culture.
Results: Of the 299 paired samples (LBC and HVS) evaluated for the presence of *Candida*, 29 LBC (9.7%) and 93 HVS (31.1%) were positive for *Candida*. The sensitivity, specificity, PPV, NPV and accuracy of the LBC was 25.8, 97.6, 82.8, 74.4 and 75.3% respectively. The LBC and HVS showed a fair agreement in detection of *Candida* (Kappa value = 0.307, p value < 0.05).
Conclusions: 1. LBC is 26% sensitive and 75% accurate; therefore, it can assist but is not always reliable for diagnosing of vaginal Candidiasis. 2. The performance of LBC in the detection of vaginal Candidiasis is similar to that of conventional Pap smears.

Keywords: sabouraud dextrose agar, liquid based cytology, candida species, high vaginal swab.

1. Introduction
The primary role of the LBC is the detection of atypical epithelial cells in the cervix [1]; however, it can play a marginal role in the diagnosis of infections such as Human Papillomavirus (HPV), *Candida* species, *Trichomonas vaginalis*, bacterial vaginosis, Cytomegalovirus and Herpes Simplex Virus (HSV) [2].
The significance of a *Candida* infection in an individual depends on the immune status of the patient [2]. In immunocompetent patients, *Candida* is regarded as harmless pathogen; however, in immunocompromised individuals, it is regarded as a harmful organism that can lead to systemic mycosis [3].
Candida infection can lead to an inflammatory Pap smear characterized by abundant neutrophils which are capable of masking atypical epithelial cells [4, 5]. *Candida* has two main types (*C. albicans and C. glabrata*) that infect the vagina, vulva and cervix resulting in a condition known as vulvovaginal candidiasis [4]. Vulvovaginal candidiasis is characterized by a burning sensation, itchiness, and a whitish cheesy discharge [6]. However, about 40% of the cases are asymptomatic [7].
In as much as LBC plays a role in the diagnosis of *Candida* infections of the female genital tract, culture of a High Vaginal Swab (HVS) on Sabouraud Dextrose Agar (SDA) is the gold standard [8]. Sabouraud Dextrose Agar is classified as a selective medium for the isolation of fungi from clinical specimens [9].

The selective behavior of SDA is due to its acidic pH (about 5.0) which suppresses the growth of bacteria while permitting the growth of fungi [9]. In addition, antibacterial drugs such as Chloramphenicol, Tetracycline and Gentamicin may be added to the agar to inhibit overgrowth of bacteria [9]. After 48 hours of incubation, *Candida* is identified as white or creamy colonies [9]. *Candida albicans* can further be confirmed by a germ tube test [9]. In a stained LBC slide, *Candida* can be identified in the spore or pseudohypheal form [4].

Both forms are usually eosinophilic and are usually found interspersed with squamous cells [6]. The yeast is usually budding and has an approximate diameter of 3-7 *u*m [4]. The pseudohyphae usually show constrictions along their length [4]. In liquid based preparations, pseudohyphae may cause squamous cells to have a linear arrangement (spearing of epithelial cells) [6]. This is known as the *'shish kebab'* effect [6]. Tangles of pseudohyphae ("spaghetti") and yeast (meat balls) may occasionally be found together [4]. *Candida* infection is usually associated with other inflammatory changes such as keratotic change and numerous fragmented leukocytic nuclei (polydust) [4]. Some intermediate cells may show perinuclear degenerative vacuoles which mimic koilocytes [4]. *Candida* induced inflammation causes slight nuclear enlargement which can easily result in an atypical squamous of undetermined significance (ASCUS) interpretation [6].

At Cimas Medical laboratories, both LBC and High Vaginal Swabs (HVS) are processed. However, there is uncertainty regarding of the sensitivity, specificity, and accuracy of LBC in the diagnosis of *Candida* infections of the vagina. In addition, there is paucity of published data on the performance of LBC in the diagnosis of vaginal Candidiasis. This study, therefore, sought to determine the sensitivity, specificity, NPV, PPV and accuracy of LBC in detecting vaginal *Candida* infections in patients presenting with vaginal discharge in comparison with HVS culture.

2. Material and Methods

2.1 Study Design: Cross-sectional descriptive study from January 2020 to May 2021.

2.2 Study Sites: Cimas Medical Laboratories**,** Harare, Zimbabwe.

2.3 Study Population: Women presenting with vaginal discharge.

2.4 Study Entry Criteria: Women of all ages. Women already on antifungals before sample collection were excluded from the study.

2.5 Sampling Method: Consecutive sampling method

2.6 Sample size: A total of 299 paired samples (cervical LBC and HVS) from women presenting with vaginal discharge on clinical examination were prospectively analyzed in this study. All 299 paired samples were from different women.

2.7 Study objectives

1. To determine the sensitivity, specificity, NPV, PPV, and accuracy of LBC in the diagnosis of vaginal Candidiasis.
2. To determine the level of agreement between LBC results and the HVS culture on SDA agar (gold standard).

2.8 Sample processing

2.8.1 Liquid Based Cytology samples

A ThinPrep 2000 machine (Hologic Inc – Marlborough, MA 01752, USA) was used to deposit a monolayer of cells on to a ThinPrep charged microscopy slides (Hologic Inc – Marlborough, MA 01752 USA). The LBC slides were stained using the Papanicolaou stain and evaluated for the presence of *Candida* using a microscope.

2.8.2 High Vaginal Swabs samples

HVS samples were cultured on an SDA agar with Chloramphenicol. After 48 hours of incubation, positive cultures had a growth of creamy colonies. A germ tube test was used to differentiate *Candida albicans* from other *Candida species.*

2.8.3 LBC slides interpretation

The LBC slides were analyzed prospectively by two primary independent individuals, a Cytologist (MSc Clinical Cytology) and a pathologist (MMED Anatomic Pathology). Discrepant findings were referred to a third person, a pathologist (MMED Anatomic Pathology). All personnel were blinded of the HVS results on the first analysis. All HVS culture positive patients had their LBC slides reviewed to ensure that they were truly negative.

2.9 Data analysis and Data presentation

All data was analyzed using SPSS version 25. All statistical tests were performed at 5% level of significance. A Cohen's Kappa test was done to determine the level of agreement between LBC and HVS culture. The LBC and HVS results were compared using established formulae to determine the sensitivity, specificity, accuracy, PPV and NPV. Descriptive statistics were presented in the form of tables, charts, and graphs.

2.10 Ethical Approval

Ethical approval was obtained from the Ethical Review Board, certificate number: 99/2019. Permission was also granted by Cimas Medical laboratories for access to patient data. During the study, strict patient confidentiality was observed.

3. Results and Discussion

A total of 299 study participants had their paired samples (HVS and LBC) evaluated for the presence of *Candida.*

3.1 Age characteristics of study participants

The mean (SD) age of the participants was 36.4 (9.5) years and the age range were 18-75 years. Figure 1 below shows that vaginal discharge peaked in the age group 31-40 years.

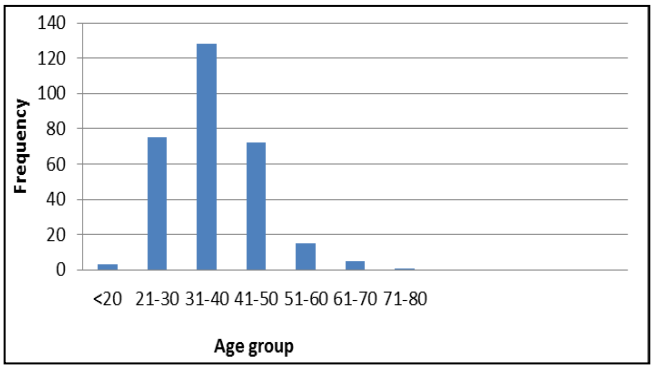

Fig 1: Age characteristics of study participants.

3.2 Study findings
Of the 299 paired samples evaluated for the presence of *Candida*, 29 LBC smears (9.7%) were positive and 93 HVS samples (31.1%) were positive for *Candida*. Fifty-six (60.2%) of the detected *Candida* were of the *C. albicans* type and 37 (39.8%) were other *Candida species.*

3.3. Sensitivity, Specificity, PPV, NPV, and accuracy of LBC for the detection of the Candida.
The sensitivity, specificity, PPV and NPV of the LBC for the detection of *Candida* was 25.8, 97.6, 82.8, 74.4% respectively. The overall accuracy of the Pap smear was 75.3% in this study.

3.4. Correlation of LBC and HVS results
Table 1 below shows the cross tabulation of LBC and HVS culture findings. LBC and HVS showed a fair agreement (Kappa value=0.307, p value < 0.05).

Table 1: Cross tabulation of LBC and HVS culture findings

		HVS culture results		Total	Kappa value	p-value
LBC results		Positive	Negative		0.307	<0.05
	Positive	TP =24	FP=5	29		
	Negative	FN=69	TN=201	270		
Total		93	206	299		

LBC: Liquid Based Cytology, HVS: High Vaginal Swab, TP: True Positive, FP: False Positive, TN: True Negative.

3.5. Discussion
The Papanicolaou stain is the most common cytological stain[6]. It is a polychromatic stain which is composed of a combination of hematoxylin stain, Orange G 6 (OG6) stain and Eosin Azure (EA) stain[4]. It differentially stains cellular cytoplasm according to their maturity [6]. Old cells are stained with OG6 and youthful cells are stained predominantly by EA [6]. In addition, the Pap stain also stains extracellular components such as mucin, crystals, fungi, and bacteria [6].

In this study, the patient age ranged from18-75 years. The range was wider than the one used by Avwioro *et al*. who excluded patients below the age of 20 years [11]. The reason for the exclusion of women below 20 years in that study was their difficulty in finding women who came for cervical cancer screening at that age and therefore they could not get LBC samples. In this study, 3 (1%) of the patients were below 20 years old. The prevalence of vaginal discharge peaked in the age group 20-39 years which was consistent with findings by Avwioro *et al*. [11].

In this study, *Candida* was diagnosed in 29 (9.7%) (LBC) and 93 (31%) (HVS culture). LBC findings were slightly higher than findings by Avwioro *et al*. [11] which recorded 7.6% (Conventional Pap smears) because LBC produces clearer smears than Conventional Pap smears resulting in easier identification of fungal elements [1]. HVS culture results of this study were, however, comparable with those of Avwioro *et al*. which had 31.1% [11]. Findings of both studies were; however, lower than the 43% (culture) reported by Donders *et al*. [12]. The difference could be attributed to the use of pregnant women as the study population in the Donders *et al*. study. Pregnant women are prone to immunosuppresion compared to non-pregnant women. In another study that used pregnant women as a study population, Alteras *et al*. recorded a low detection rate of 14% (culture) [13]. The difference between these two studies can possibly be explained by different accessibility to antifungals before culture samples were collected. The sensitivity of LBC for the detection of *Candida* in this study (25.8%) was comparable to the sensitivity of a conventional Pap smear recorded by Avwioro *et al*. (25.3%) [11].

In this study, *C. albicans* was the dominant type detected in LBC samples with 56 cases (60.2%). *Candida* other than *C. albicans* were detected in 37 cases (39.8%). These findings were in keeping with findings by Donders *et al*. [12]. However, Donders *et al*. were fortunate to be able to further characterize the other *Candida* further into *C. glabrata, C. tropicalis* and *C. krusei* unlike in this study [12].

Cohen's Kappa test was done to determine the level of agreement between LBC and HVS culture for the detection of *Candida*. The test yielded a fair agreement (Kappa value = 0.307, $p<0.05$). Avwioro *et al*. reported that mild *Candida* infection can easily be missed in Pap smears [11]. This could have contributed to the fair agreement.

4. Conclusions
1. LBC is 26% sensitive and 75% accurate; therefore, it can assist but is not always reliable for diagnosing of vaginal Candidiasis.
2. The performance of LBC in the detection of vaginal Candidiasis is similar to that of conventional smears.

5. Acknowledgements
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6. Conflict of Interest: None

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