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Colour Speaks: Decoding Vaginal Health with pH Responsive Strip.

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ABSTRACT: -

The vaginal pH is a critical biomarker reflecting the physiological and microbial status of the vaginal milieu. Normal vaginal pH (3.8–4.5) is sustained by lactic acid-producing Lactobacillus species, and deviation from this range is indicative of pathophysiological conditions such as bacterial vaginosis, vulvovaginal candidiasis, or trichomoniasis. pH-sensitive vaginal diagnostic strips offer a rapid, non-invasive screening modality for the assessment of vaginal acidity. These strips, formulated using inert biopolymeric substrates embedded with pH-indicating chromophores, undergo a distinct colorimetric transition upon exposure to vaginal secretions. Their integration into self-diagnostic tools enhances early detection and pharmacological intervention in vaginal infections, thereby reducing disease burden and improving reproductive health outcomes.

Keywords: - female intimate hygiene, female intimate hygiene, intimate washes/ cleansing products, microbiota, vulvovaginal.

1.Introduction

The vagina functions as an external communicative conduit of the female reproductive system, facilitating the excretion of menstrual fluid and serving as the birth canal during parturition. It harbours a distinct microbial community, predominantly composed of Lactobacillus species, which plays a crucial role in preserving the local biochemical and physiological milieu. The stability of this microbiota is dependent on the dynamic equilibrium of multiple ecological factors, including pH, hormonal levels, and host immune responses. Disruption of this balance can predispose the vaginal environment to pathogenic colonization and infection. {1} The healthy vaginal epithelium is coated with a thin layer of clear mucosal secretion, referred to as vaginal fluid, which plays a 3variety of endogenous and exogenous factors, including microbial infections, senescence-related hormonal changes, sexual intercourse, and the use of intravaginal cleansing agents such as douches. [2,3] Lactobacillus species, which dominate the normal vaginal microbiota, play a key role in maintaining vaginal health by producing lactic acid and bacteriocins. These substances help sustain an acidic environment that inhibits the growth of harmful microorganisms. This acidic pH acts as a natural defense mechanism, protecting the vaginal tract against sexually transmitted infections and opportunistic pathogens. [4] The depletion or significant reduction of commensal vaginal microbiota, particularly *Lactobacillus* species, disrupts the microbial equilibrium of the vaginal ecosystem, thereby facilitating the overgrowth of opportunistic or pathogenic microorganisms, a condition commonly leading to vaginitis. In 2011, Ravel et al. classified the vaginal microbiome into five distinct Community State Types (CSTs), providing a foundational framework for understanding microbial composition and its association with vaginal health and disease. [5] This

classification system offers a robust framework for characterizing the human vaginal microbial community (HVMC). The microbial communities are categorized into five Community State Types (CSTs): four of which are dominated by specific Lactobacillus species—L. inners, L. cristatus, L. gasser, or L. Jesenia. The fifth CST is characterized by a diminished presence of lactic acid-producing bacteria and an increased abundance of obligate anaerobic microorganisms. Vaginal dysbiosis associated with these shifts may predispose individuals to various forms of vaginitis, including bacterial vaginosis (BV), vulvovaginal candidiasis, trichomoniasis, and aerobic vaginitis.[6]. This review primarily addresses the three most prevalent forms of vaginitis. Currently, the clinical diagnosis of vaginitis relies on established criteria based on characteristic signs and symptoms. Specifically, the diagnosis of bacterial vaginosis (BV) has traditionally been guided by the Amsel Criteria, which have been employed in routine clinical practice since 1983. According to these criteria, a diagnosis of BV is confirmed when at least three of the following four findings are present: (1) homogeneous, thin, grayish-white vaginal discharge; (2) elevated vaginal pH greater than 4.5; (3) a positive amine (whiff) test upon addition of 10% potassium hydroxide (KOH); and (4) the presence of clue cells—vaginal epithelial cells heavily coated with bacteria—comprising at least 20% of cells observed under microscopic wet-mount examination [7]. The Nugent scoring system is a standardized diagnostic method used to assess bacterial vaginosis by quantifying the relative abundance of specific bacterial morphotypes in Gram-stained vaginal smear specimens. The score is based on the presence or absence of large Gram-positive rods (Lactobacillus spp.), small Gram-variable rods (Gardnerella vaginalis and Bacteroides spp.), and curved Gram-negative or Gram-variable rods (Mobil uncus spp.), allowing for an objective evaluation of the vaginal microbial composition. [8]. In both of the *and distinctive biomarker for the identification of vaginitis. Deviations from the normal acidic pH range are strongly associated with an increased risk of vaginal infections. Consequently, pH measurement has been widely utilized as a preliminary screening tool for the early detection of vaginitis [9]. According to previous studies, a vaginal fluid pH within the range of 4.0 to 4.5 or lower is indicative of a healthy vaginal environment and the absence of vaginitis. In contrast, pH values exceeding

4.5 are commonly associated with the presence of vaginitis, particularly bacterial vaginosis (BV), reflecting a disruption in the normal microbial equilibrium.[10]. In cases of *Trichomonas vaginalis* infection, the vaginal pH is often markedly elevated, frequently reaching values of 6.5 or higher, reflecting significant disruption of the normal acidic vaginal environment. [11] Vaginitis presents with a range of clinical signs and symptoms that may vary depending on the underlying etiology, guiding differential diagnosis and corresponding therapeutic interventions in clinical practice. Previous studies have demonstrated that combining vaginal pH assessment with symptomatology enhances diagnostic sensitivity, thereby improving the accuracy of vaginitis detection [12]

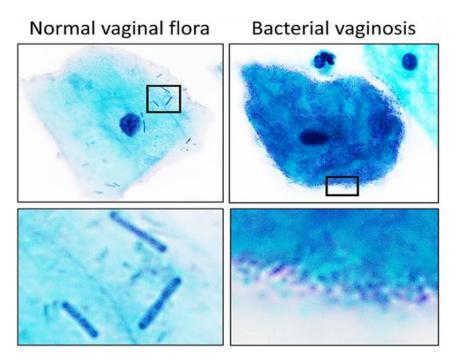
Table 1. Symptoms and signs of vaginitis (Information from [12,13,14,15,16,17]).

vaginitis	Etiology	Symptoms	Signs	Risks	pH Value
Bacterial vaginosis	Anaerobic bacteria (Prevotella, Mobiluncus, Gardnerella vaginalis, Ureaplasma, Mycoplasma)	Fishy Odor; malodorous; homogenous; clear, white, or Gray discharge that may worsen after intercourse; pelvic discomfort may be present.	No inflammation.	Increased risk of HIV, gonorrhoea, chlamydia, and herpes infections.	greater than 4.5
Vulvovaginal candidiasis	Candida albicans, Candida Kruse, Candida glabrata	No Odor; white, thick, cheesy, or curdy discharge; vulvar itching or burning.	Signs of inflammation; Vulvar erythema and oedema.	vulvodynia	4.0
Trichomoniasis	Trichomonas vaginalis	Green or yellow, frothy discharge; foul odor; pain with sexual intercourse, vaginal soreness, dysuria.	Signs of inflammation, "strawberry cervix"; Vestibular erythema may be present.	Increased risk of HIV infection Increased risk of preterm labor. Should be screened for other sexually transmitted infections.	5.0~6.0

2. Types of vaginitis

A] Bacterial Vaginosis (BV)

Bacterial vaginosis (BV) is the predominant etiologic of vaginitis in women of reproductive age, typically characterized by abnormal vaginal discharge and a malodorous Odor.[17] Bacterial vaginosis is marked by a reduction in the population of Lactobacillus species, which are essential for maintaining a healthy vaginal microbiota."[18,19]. "Bacterial vaginosis is often associated with an overgrowth of pathogenic bacteria, including *Gardnerella vaginalis*, *Atopobium vaginae*, *Megasphaera* species, *Prevotella* species, and *Sneathia* species. Diagnosis at the point of care can be established when at least three of the four Amsel criteria are met: presence of a thin, homogeneous white discharge, observation of clue cells under microscopy, vaginal pH greater than 4.5, and a characteristic fishy odor released upon addition of potassium hydroxide."[20].

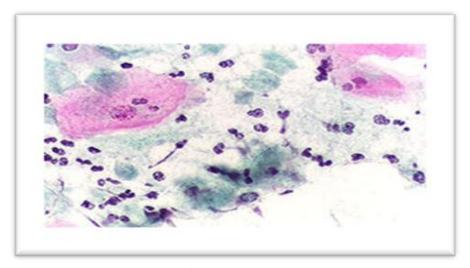


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B| Trichomoniasis

Trichomonas vaginalis is a protozoan parasite that causes trichomoniasis, recognized as the most widespread non-viral sexually transmitted infection (STI) across the globe. In the United States alone, it affects over one million individuals each year. On a global scale, the estimated number of new trichomoniasis cases reaches approximately 153 million annually. Various demographic and social factors—including age, ethnicity, income level, education, and overall socioeconomic status—play significant roles in Influencing the distribution and prevalence of this infection worldwide [21].



Pap smear, showing infection by Trichomonas vaginalis. Papanicolaou stain, 400

C] Vulvovaginal candidiasis

Vulvovaginal candidiasis (VVC) is the second most common (20-25%) cause of vaginitis after BV [22]. Vulvovaginal candidiasis (VVC) commonly results from a disruption in the balance between *Candida* species and the host's vaginal environment, often due to physiological or external factors. *Candida albicans* is responsible for approximately 85–90% of cases. However, infections caused by non-*albicans Candida* species, especially *Candida glabrata*, are becoming increasingly prevalent [23]. Most women carry Candida as normal flora of vagina without any symptoms [24]. Diagnosis of VVC is based on clinical symptoms and signs and finding yeast hyphae on potassium hydroxide microscopy. Antigen or DNA probe testing is also helpful [25].

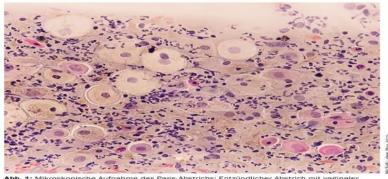


Abb. 1: Mikroskopische Aufnahme des	Paps-Abstrichs:	Entzündlicher	Abstrich mit vaginaler
Candidiasis.			

Type of Vaginitis	Treatment		
Bacterial Vaginosis	Metronidazole or clindamycin preparations		
Vulvovaginal Candidiasis	Oral fluconazole or topical antifungal agents		
Trichomoniasis	Oral metronidazole or tinidazole		
Aerobic Vaginitis	Antibacterial agents, local anti-inflammatory		
Inflammatory Vaginitis (DIV)	Vaginal antibiotics, corticosteroids, estrogen		
Atrophic Vaginitis	Vaginal estrogen formulations		
Cytolytic Vaginosis	Alkaline treatment (e.g., baking soda)		
Mixed Vaginitis	Combination therapy based on detected causes		

3. The Role of Normal Vaginal pH

The pH level specific to the vaginal environment, referred to as the vaginal pH value, serves as an important physiological indicator of vaginal health. The acidity or alkalinity of the vaginal milieu is determined by the concentration of hydrogen ions, quantified using the pH scale. While a neutral pH is defined as 7.0, the normal vaginal pH typically ranges from 3.8 to 5.0, reflecting a moderately acidic environment essential for maintaining microbial homeostasis and inhibiting the proliferation of pathogenic organisms.[2]. A vaginal pH that is lower (i.e., more acidic) than that of blood or interstitial fluid provides a protective barrier for the vaginal mucosa by creating an inhospitable environment for the colonization and proliferation of pathogenic microorganisms[4]. The relatively acidic pH of the vaginal environment, which is lower than that of blood and interstitial fluids, functions as a crucial innate defence mechanism. This acidic milieu inhibits the colonization and growth of pathogenic microorganisms, thereby contributing to the protection of the vaginal mucosal barrier and the maintenance of microbial homeostasis.[11]. The vaginal pH plays a critical role in maintaining vaginal health, and its regulation is closely linked to the metabolic activity of Lactobacillus acidophilus and other commensal microbiota, as well as the influence of endogenous factors such as estrogen levels, glycogen availability, and the composition of resident flora and potential pathogens. A dynamic interplay exists among these elements and their metabolic by-products, which collectively contribute to the maintenance of an acidic vaginal environment and microbial equilibrium.[13]. Vaginal microorganisms serve as key regulators of the vaginal ecosystem, with Lactobacillus acidophilus recognized as the predominant species contributing to its stability. This microorganism metabolizes glycogen—originating from the desquamation of estrogen-stimulated, glycogen-rich vaginal epithelial cells—into lactic acid, thereby acidifying the local environment through the release of hydrogen ions.[14]. The metabolic activity of Lactobacillus species results in the production of lactic acid, leading to an acidic vaginal pH typically ranging from 4.0 to 4.5. This acidic environment exerts a protective effect by creating a biochemical barrier that inhibits the overgrowth of pathogenic microorganisms. Disruption of this microbial equilibrium may result in abnormal vaginal pH levels, which can serve as a diagnostic indicator for the presence of bacterial pathogens and may also reflect hormonal changes associated with menopausal status.[15]. In addition, studies have confirmed that an increase in vaginal pH may lead to bacterial vaginosis (BV) and spontaneous preterm deliveries (PD) in pregnant women [16]. Based on the above research, we know that vaginal pH value has a profound impact on women's lives. Monitoring that pH level, even with self-testing, can be used to effectively manage and prevent infection.[16]

2.MATERIALS AND METHODS

1. Vaginitis test products

pH- Sensitive Paper Based Strip for detection of vaginal Infection

2Principle of pH sensitive detection

Vaginal infections such as **bacterial vaginosis (BV)** and **trichomoniasis** often cause a rise in vaginal pH, typically above **4.5**, whereas a healthy vaginal microbiome, predominantly sustained by **Lactobacillus** species, maintains an acidic environment with a pH between **3.8 and 4.5**.

To detect these pH alterations, **paper-based strips** embedded with **acid-base indicator dyes** are used. These indicators respond to the hydrogen ion concentration in vaginal secretions by changing color, offering a **simple**, **colorimetric method** to visually assess abnormal pH levels indicative of possible infections.[26].

5. Formulation-

Materials required:-

Component	Purpose	
Whatman filter paper (No. 1) or cellulose- based paper	Serves as the base substrate for the pH strip, allowing uniform absorption of reagents.	
pH-sensitive dyes(e.g., Bromocresol purple, Bromothymol blue)	Act as colorimetric agents to detect pH changes corresponding to vaginal conditions.	
Natural polymer binders such as chitosan or gelatin	Used to entrap and stabilize the dye on the paper matrix, improving durability and preventing dye leaching.	
Citric acid	Functions as a crosslinking agent to enhance the adhesion and stability of the polymer-dye matrix.	
1% Acetic acid	Used as a solvent medium for dissolving chitosan during binder preparation.	
Ethanol or distilled water	Acts as the primary solvent for dissolving and dispersing dyes and other reagents.	
Standard lab tools (Petri dishes, beakers, forceps)	Required for solution preparation, soaking, and handling of strips.	
Drying apparatus (e.g., hot air oven or incubator)	Facilitates rapid and consistent drying of the coated strips under controlled conditions.	

Sharma, S. et al. (2021). Development of polymer-supported paper-based colorimetric biosensors. Biosensors and Bioelectronics, 171, 112732.[27]

- 1. Step:-1
- Prepare dye solution
- Weigh 0.1 grams of Bromocresol Green and dissolve it in 50 mL of ethanol.
- Separately, dissolve 0.05 grams of Phenol Red in 25 mL of ethanol.
- 3. Combine both dye solutions thoroughly to prepare a total of 75 mL of indicator mixture
- 2. Step:-2
- Cut filter paper into strips
- 1.Use a pair of scissors or a precision cutter to trim the paper into uniform strips measuring approximately 0.5 cm in width and 5 cm in length.
- 2.Handle using forceps or gloves to avoid contamination.

3. Step:-3

- Dye impregnation
- Immerse paper strips into the indicator mixture for 10 minutes.

Use forceps for easy handling and uniform immersion.

4.Step4:-

- Dry the strips
- Lay the soaked strips on a clean, dust-free surface in a flat position.

Allow them to air dry in a dark environment for 12 to 24 hours to prevent light-induced degradation .

Alternatively, the strips can be dried in a hot air oven set to 40°C for 30 to 60 minutes for faster processing.

5. Step 5:-

- Calibration
- Prepare buffer solution of known pH [3.0,4.0,4.5,5.0,6.0,7.].
- Deep a strip into each buffer.
- Record the colour change of each pH.
- Use the data to create a colour comparison chart.

6. Step 6:-

- o Packaging and storage
- Once fully dried, store the strips in sealed, light-resistant pouches along with a desiccant such as silica gel to prevent moisture absorption.
 Include a reference color chart to enable accurate interpretation of pH changes during use. [28,29,30].

6. Utilization method

1. Hand Hygiene

 Begin by washing your hands thoroughly with soap and water. This helps reduce the risk of introducing contaminants during the testing process.

2.Find a Comfortable Position

 Choose a position that allows easy access to the vaginal area. You may sit on the toilet, squat, or stand with one foot placed on a raised surface such as a toilet seat or low stool

3. Sample Collection Methods

Depending on the test strip design, use **one of the following methods** to obtain a vaginal fluid sample:

4.Method A: Direct Strip Application

- Put on disposable gloves.
- Gently insert the reactive end of the strip approximately 2 to 3 centimeters into the lower vaginal area.
- \circ Keep the strip in place for **5 to 10 seconds**, allowing it to absorb vaginal secretions.
- Carefully remove the strip without letting it touch other surfaces.

5.Method B: Swab Transfer Technique

- Using a sterile cotton swab, gently insert it into the vaginal canal and rotate it for 5 to 10 seconds to collect a sample.
- Withdraw the swab and immediately press it onto the test area of the strip for about 10 seconds.
- o Avoid using too much fluid, as excess moisture can lead to inaccurate color readings.

6 Wait and Observe the Reaction

- O Allow the strip to sit undisturbed for 30 to 60 seconds after sample application.
- Examine the test area to observe any color change, which indicates the pH level of the sample.

7. Dispose Properly

o Discard the used strip and gloves into a covered bin.

Wash your hands thoroughly.

7.Important Considerations

- **Do not use** during menstruation or immediately after intercourse.
- Avoid testing within 24 hours of using vaginal products (douches, creams, etc.).
- This is a screening tool, not a definitive diagnosis.
- Follow up with a healthcare provider if symptoms persist or results are abnormal.



well Health: How to Use At Home Vaginal pH Tests Winx Health+12Verywell Health+12Intimate Rose+12Vivoo+2Amazon+2Amazon+2

8.Advantages

- 1. Economical:- Produced using low-cost components such as filter paper and pH indicators, making them suitable for widespread distribution, especially in low-resource areas.
- 2.Use friendly:- Simple to operate without the need for technical expertise; can be used independently at home with basic guidance.
- 3,Quick feedback:- Delivers results within a minute, facilitating timely evaluation and potential follow-up..
- 4. Minimally Inavasive:- Sample acquisition involves little to no discomfort, either through gentle insertion or swabbing.
- 5.Highly portable:-Lightweight and compact design enables easy storage and use in community or remote health programs.
- 6.Instrument free operation:- Does not require any electrical devices or lab tools, ideal for field testing and rural clinics.
- 7.Early detection acid:- Supports early recognition of vaginal pH changes, potentially preventing complications from progressing.
- 8. Visual and intuitive readout:- Provides a distinct color shift that corresponds to pH levels, allowing straightforward comparison with a reference chart.
- 9. Support infection deffrentiation:- Helps distinguish between types of infections; for example, elevated pH may signal bacterial or parasitic infections, while acidic pH usually aligns with yeast infections
- 10.Promotes personal health monitoring:-Encourages proactive health care, particularly for women in regions with limited access to gynaecological services.[36]

9. Disadvantages

1. Not Diagnostic Alone

pH strips cannot identify the specific cause of vaginal imbalance. Additional tests are required to confirm infections.

2. Ineffective for Yeast Infections

pH often remains normal (<4.5) in cases of Candida infections, which makes the strip unreliable for yeast detection.

3. Potential for Misinterpretation

Results depend on color changes, which can be subjective and difficult to interpret correctly without adequate training or color vision.

4. Influenced by External Factors

Activities like douching, recent intercourse, menstruation, or using lubricants can skew pH levels and cause false readings.

5. Storage and Shelf-Life Issues

Strips may degrade over time or under improper storage conditions, leading to inaccurate results.

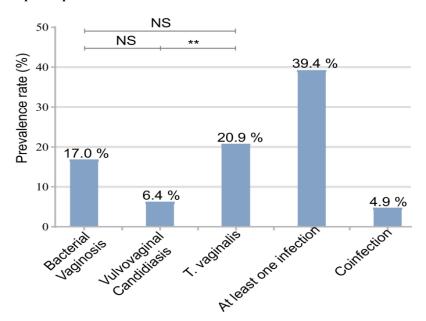
6. No Detection of Other Markers

Does not test for key indicators like amines, leukocytes, or proteins that may also be relevant for comprehensive diagnosis.

7. May Cause Mild Discomfort

Some users may feel discomfort inserting the strip or using a swab, especially during pregnancy or menstruation[37,38,39,40,]

10. Accuracy of virginial pH strips



The prevalence and risk factors of Trichomonas vaginalis in Wuhan and the Tibetan area, China: a two-center study[41].

Summary

Vaginal health is a critical aspect of women's overall well-being, with disturbances potentially leading to broader health implications. Among various biomarkers, **vaginal pH serves as a vital indicator**, significantly influencing the diagnosis, monitoring, and management of vaginitis and related conditions. Multiple internal and external factors can alter vaginal pH, contributing to different forms of vaginitis—each presenting with distinct symptoms, risks, and treatment approaches.

Recurrent vaginitis has also been linked to underlying immune system dysregulation. In this context, vaginal pH self-testing tools have emerged as accessible, low-cost methods for early detection and surveillance of pH imbalances. These tests, when combined with clinical evaluation or other diagnostics, offer a practical solution both for in-clinic use and for self-monitoring at home. Their integration into routine care may promote greater awareness and proactive management of vaginal health.

Result and Discussion

pH-sensitive vaginal strips represent a cost-effective, user-friendly method for assessing vaginal health, especially for identifying conditions like **bacterial vaginosis** and **trichomoniasis**, both of which are typically linked to elevated vaginal pH. These diagnostic tools deliver quick, visual results and can be utilized in both clinical environments and for home use, making them especially beneficial in **low-resource or rural settings**.

Although not intended to replace comprehensive laboratory diagnostics, these strips can greatly support early detection efforts when interpreted alongside clinical symptoms or medical history. They also play a valuable role in **promoting self-monitoring and awareness** among women regarding their reproductive health.

Despite their advantages, some limitations persist. These include **lower sensitivity in identifying yeast infections**, which generally do not cause pH changes, and the possibility of **inconsistent results due to improper usage**. Ongoing development may lead to more advanced versions—such as **multiplexed pH strips or digital integrations** with smartphone-based apps—to enhance diagnostic precision and data tracking.



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