The epidemiology, pathogenesis, and diagnosis of vulvovaginal candidosis: A mycological perspective

Macit Ilkit¹ and Ahmet Baris Guzel²

¹Division of Mycology, Department of Microbiology, Faculty of Medicine, University of Çukurova, Adana, Turkey, and ²Department of Obstetrics and Gynecology, Faculty of Medicine, University of Çukurova, Adana, Turkey

Abstract
Vulvovaginal candidosis (VVC) is the second most common cause of vaginitis after bacterial vaginosis, and it is diagnosed in up to 40% of women with vaginal complaints in the primary care setting. Reliable diagnosis of VVC requires a correlation of clinical features with mycological evidence. The mycological methods used for diagnosis include microscopic examination, fungal culture, and antigen tests. Fungal culture can reveal the species of organism(s) responsible for the infection and provide epidemiological data. This report reviews current knowledge about the available diagnostic methods and tests that accurately diagnose VVC, and highlights the importance of fungal culture.

Keywords: Candida albicans, chromogenic media, immunochromatography, microscopic examination, polyfungal, vaginitis

Introduction
Infectious vaginitis is among the most common reasons that women seek gynecological care and includes three types of vaginal infections: bacterial vaginosis (BV), candidosis, and trichomoniasis (TR), with prevalence rates of 22–50%, 17–39%, and 4–35%, respectively (Nyirjesy and Sobel, 2003; Anderson et al., 2004). Vaginal Candida infections are commonly referred to as “Candida vaginitis” or “vulvovaginal candidiasis” (VVC) (Sobel et al., 1998). VVC is the second most common cause of vaginitis after BV and is diagnosed in up to 40% of women with vaginal complaints in the primary care setting (Nyirjesy and Sobel, 2003; Anderson et al., 2004). Yeast of the genus Candida are commensal organisms that colonizes the skin, the gastrointestinal tract, and the reproductive tract. The presence of Candida in the vagina in the absence of immunosuppression or damaged mucosa is usually not associated with any signs of disease: this type of colonization is referred to as asymptomatic colonization. VVC, however, is defined as signs and symptoms of inflammation in the presence of Candida spp. and in the absence of other infectious etiologies (Sobel et al., 1998; Sobel, 2007). In this review, PubMed (Medline) was searched for clinical and mycological studies published in English (up to February 2011) using the key words “vulvovaginal candidiasis,” “vulvovaginal candidosis,” and “Candida vaginitis.” The goal of this review is to provide an update on the diagnostic methods for VVC for microbiologists and clinicians in the field. Throughout this report, laboratory diagnoses of VVC based on determinations of vaginal pH, microscopic examinations, fungal culture, and antigen tests are reviewed. In addition, signs and symptoms of VVC with clinical manifestations, pathogenesis of the infection, and epidemiological characteristics are highlighted. However, virulence factors of Candida spp., predisposing factors, VVC immunology, and antifungal treatment modalities will not be addressed in this review.

Vaginal flora
The vaginal ecosystem is complex and includes several microorganisms that are difficult to cultivate and identify. Therefore, these organisms are unfamiliar to many microbiologists and gynecologists (Hillier et al., 1993). The microorganisms that colonize the vagina fluctuate with a woman’s age, hormonal and immune status, sexual activities, use
of medications, mode of contraception, and exposure to a variety of vaginal products (Ledger and Witkin, 2007). Facultative lactobacilli regulate the normal vaginal flora, comprising 50–90% of the aerobic vaginal flora in women (Eckert, 2006), and are usually present at concentrations of $10^7$–$10^9$ cfu/g of vaginal fluid (Redondo-Lopez et al., 1990). In addition, lactobacilli are known to produce antibacterial compounds including lactocidin, acidolin, lactacin B, and hydrogen peroxide ($H_2O_2$) (Hillier et al., 1993). Lactobacilli metabolize glycogen and other components in vaginal secretions and produce lactic acid, which lowers vaginal pH, and they have long been considered to constitute the primary microbiological barrier against infection by genital pathogens (Redondo-Lopez et al., 1990).

It has been postulated that the normal vaginal bacterial flora, especially *Lactobacillus* species, play a critical role in the prevention of bacterial vaginal infections and the transmission of pathogenic organisms (Sobel and Chaim, 1996). Specifically, *L. crispatus*, *L. jensenii*, and *L. gasseri*, but not *L. acidophilus*, are the predominant lactobacilli found in normal vaginal flora. The majority of *L. crispatus* and *L. jensenii* clinical isolates appear to produce $H_2O_2$, a product with potent antimicrobial activity in vitro (Antonio et al., 1990; Ledger and Witkin, 2007). Growth inhibition of one bacterial species by the $H_2O_2$ generated by another species is a well-recognized mechanism of bacterial antagonism (Eschenbach et al., 1989). Although lactobacilli are the predominant bacteria in the vagina, other bacteria are also present, including streptococcal species, Gram-negative bacteria, anaerobes, and *Gardnerella vaginalis* (Eckert, 2006). Notably, *Candida* species comprise a portion of the lower genital tract flora in approximately 20% (range 10–80%) of healthy asymptomatic women (Goldacre et al., 1979; Sobel, 2007). The production of $H_2O_2$ and the maintenance of a normal pH by *Lactobacillus* species are protective against invasion or overgrowth of pathogenic species and are thought to reduce the incidence of BV (Auger and Joly, 1980; Sobel and Chaim, 1996). However, similar conclusions related to VVC remain controversial (Sobel and Chaim, 1996; McClelland et al., 2009; Zhou et al., 2009).

Molecular methods based on the analysis of 16S and 18S rRNA gene sequences directly extracted from samples not only obviate the need to cultivate organisms but also permit high-throughput analysis of samples, and provide precise and detailed information about the populations present. Such methods may help us to better understand the dynamics of vaginal microbial communities in women with frequent VVC and to establish whether fluctuations in these communities are linked to the onset of acute frequent VVC (Vitali et al., 2007; Martinez et al., 2008; Zhou et al., 2009). However, PCR has limited clinical value in distinguishing a "pathogen" from the endogenous microbial flora that exist in the genital tract. Similarly, PCR detection of *Candida* spp. in vaginal samples is possible, but this approach offers no benefit over fungal culture in asymptomatic women. It can be difficult to determine whether a positive PCR result represents colonization or a true infection that warrants treatment (Tabrizi et al., 2006). In one study, Trama et al. (2005) examined vaginal swabs from 3978 women without clinical information regarding their medical histories or clinical presentations. *Candida* DNA was detected in 1316 patients (33.1%) from the studied population. However, these authors did not have a gold standard method to assess PCR sensitivity.

Nugent et al. (1991) proposed Gram-staining for vaginal smears, which has became a standardised method for BV diagnosis. Using this method, a score of 0–3 corresponds with normal flora, a score of 4–6 corresponds with disturbed flora and is designated intermediate, and a score of 7–10 corresponds with BV. Siegler (1946) first suggested that vaginitis caused by *Candida* spp. was associated with intermediate flora patterns, and Hillier et al. (1992) then reported a similar association between VVC and intermediate flora patterns. In addition, it was noted that women with intermediate flora were more likely to have positive Amsel’s et al. (1983) criteria positive than women with normal flora, though they were less likely to have positive Amsel criteria than women with BV (Hillier et al., 1992).

Anecdotally, the reduction of lactobacilli in the vaginal tract predisposes women to VVC. In contrast, Sobel and Chaim (1996) compared the microbial flora of vaginal secretions in healthy, nonpregnant women of reproductive age with the microbial flora of vaginal secretions in women with similar characteristics with acute recurrent VVC and found that women with VVC had similar numbers of bacteria and of species of the *Lactobacillus* genus. In addition, Zhou et al. (2009) suggested that commensal vaginal bacterial species might be incapable of preventing VVC. In other studies, no consensus has been achieved regarding whether the presence of $H_2O_2$-producing lactobacilli in vaginal microbial communities protects their hosts against VVC (Hillier et al., 1993; Vitali et al., 2007; Martinez et al., 2008). In addition, studies have failed to provide evidence that an altered or abnormal vaginal bacterial flora predisposes women to recurrent episodes of VVC in the absence of antibiotic intake (Sobel and Chaim, 1996; Vitali et al., 2007; Zhou et al., 2009). In face of this view, one recent study reported that vaginal *Lactobacillus* colonization was associated with a $\geq 4$-fold increase in the likelihood of symptomatic VVC (McClelland et al., 2009).

**Vulvovaginal candidosis: an overview**

VVC results from overgrowth of various *Candida* spp. that may already be present in the vagina as commensal organisms, and symptomatic infection is correlated with a high vaginal fungal burden (Sobel et al., 1998). It is estimated that approximately three out of four healthy adult women experience at least one episode of VVC during their lives, most commonly when they are of childbearing age (Geiger et al., 1995). Nearly one in two women experiences two or more episodes during their lifetime (Carroll et al., 1973), and 5–8% of these women have recurrent VVC (RVVC) (Sobel, 1985).

VVC cases are classified either as uncomplicated (~90%) or complicated (~10%) based on clinical...
presentation, microbiological findings, host factors, and response to therapy. Uncomplicated VVC is defined as sporadic or infrequent (≤ 3 episodes per year) mild-to-moderate symptoms caused by *C. albicans*, which is responsive to all forms of antifungal therapy, including short-course therapy in immunocompetent women. Complicated VVC cases include the following: (1) cases of severe VVC, (2) VVC associated with pregnancy or other concurrent conditions, e.g., uncontrolled diabetes mellitus and immunosuppression, and (3) RVVC in immunocompetent women (Sobel et al., 1998; Sobel, 2007; Pappas et al., 2009).

RVVC is defined as ≥ 4 culture-verified symptomatic episodes of VVC within 1-year and is usually caused by azole-susceptible *C. albicans* (Sobel et al., 1998; Sobel, 2007; Pappas et al., 2009). RVVC is not considered to be a chronic infection, but rather a disease with recurrent episodes. The cause of these recurrences is unknown in the majority of women (Márdh et al., 2002; Nyirjesy and Sobel, 2003). In addition, *Candida* species other that *C. albicans*, in particular *C. glabrata*, often cause RVVC (Sobel et al., 1998; Nyirjesy and Sobel, 2003; Anderson et al., 2004; Richter et al., 2005; Sobel et al., 2007). It is important to note that RVVC refers to clinical situations in which the host, microorganism, and information related to the possible usage of antifungal therapy must all be considered before a therapeutic regimen is established to ensure a successful outcome (Sobel et al., 1998).

The proportion of women with VVC who have been identified as harboring *Candida* spp. varies widely, depending on the country, region, and population (Nyirjesy et al., 1995; Anson and Allen, 1997; Chaim, 1997; Spinillo et al., 1997; Okungbowa et al., 2003; Richter et al., 2005; Guzel et al., 2011). Recently, we noted that only half of the vaginal cultures in Adana, Turkey (50.4%), were positive for *C. albicans* (Guzel et al., 2011). This percentage is substantially lower than the values typically reported in studies conducted in Europe (Anson and Allen, 1997; Spinillo et al., 1997; Ozcan et al., 2010) and North America (Nyirjesy et al., 1995; Richter et al., 2005). The rates of *C. albicans* isolates in the acute and recurrent forms of VVC and in controls (a mixed group of asymptomatic patients with no history of RVVC and some control patients who were positive for *Candida* spp.) were 37.3%, 54.2%, and 8.5%, respectively, whereas the non-*C. albicans Candida* vaginitis rates in these groups were 24.1%, 59.9%, and 25%, respectively (Guzel et al., 2011). In data from five independent studies, the overall percentages of non-*C. albicans Candida* isolates were 12% in England (Anson and Allen, 1997), 17% in Italy (Spinillo et al., 1997), 24% in the United States (Richter et al., 2005), 42.9–49.6% in Turkey (Ozcan et al., 2010; Guzel et al., 2011). In addition, episodes of VVC due to non-*C. albicans Candida* appear to be increasing in frequency (Vermitsky et al., 2008). Briefly, Vermitsky et al. (2008) analyzed 93,775 vaginal samples in the United States that were suspected of having VVC using *Candida* species-specific PCR tests over a 4-year period. They found that, with age the prevalence of non-*C. albicans Candida* species (e.g., *C. glabrata* and *C. parapsilosis*) increased, while the prevalence of *C. albicans* decreased ($P \leq 0.05$).

Three crucial factors contribute to the increased incidence of non-*C. albicans Candida* vaginitis: (1) when treating patients with RVVC, clinicians almost never perform a vaginal culture to identify the species in yeast isolates before treatment and, therefore, overlook and ignore cases of *C. glabrata*, which require a different and unconventional antifungal treatment, e.g., boric acid (Chaim, 1997; Sobel and Chaim, 1997), (2) the widespread and free over-the-counter (OTC) administration of antifungal drugs, in particular topical or oral azoles, eliminates more sensitive *C. albicans* and selects for more azole-resistant non-*C. albicans Candida* species (Chaim, 1997), and (3) the increased use of long-term maintenance antifungal regimens aimed at preventing recurrences in women (Sobel and Chaim, 1997). However, an increase in resistance to fluconazole maintenance has yet to be observed.

Overall, 70–90% of VVC cases are caused by *C. albicans*, while infections caused by *C. glabrata* are found in 10–20% of women (Sobel et al., 1998; Nyirjesy and Sobel, 2003; Sobel, 2007). Vaginitis is infrequently caused by *C. krusei*, *C. parapsilosis*, and *C. tropicalis*, although most species of *Candida* have been associated with these infections (Sobel, 2007). For example, a possible variant of *C. albicans* known as *C. africana* has been recovered mainly from patients with VVC (Tietz et al., 2001). In addition, *C. stellatoidea*, another variant of *C. albicans*, has been isolated from a number of clinical sources, but most frequently from the human vaginal tract (Kwon-Chung et al., 1990).

### Clinical manifestations of vulvovaginal candidosis

The diagnosis of VVC begins with obtaining a patient history and searching for clues. Physical examination is important not to confirm the diagnosis of VVC but to suggest alternative diagnoses (Ledger and Witkin, 2007). Although it is not associated with any mortality, VVC and RVVC are associated with considerable morbidity (Anderson et al., 2004; Eckert, 2006). Symptoms of vaginitis can cause substantial distress, resulting in time lost from work, and altered self-esteem (Eckert, 2006). Thus, it is not surprising that vaginal complaints are the most common reason for gynecological consultation (Anderson et al., 2004). However, Schwiertz et al. (2006) reported a 77% rate of misdiagnosis of VVC by physicians based on clinical evidence alone. Approximately 30% of symptomatic women remained undiagnosed after clinical evaluation (Anderson et al., 2004). More recently, Lowe et al. (2009) compared the accuracy of the clinical diagnosis of vaginitis with DNA analysis of vaginal fluid. For each infection, a clinical diagnosis demonstrated a sensitivity and specificity of 80.8% and 70.0%, respectively, for BV, 83.8% and 84.8%, respectively, for VVC, and
84.6% and 99.6%, respectively, for TR (compared with the DNA probe, which is recognised as the gold standard).

The symptoms of VVC and BV are itch and odor, respectively, with almost no overlap between the two (Eckert et al., 1998; Anderson et al., 2004; Sobel, 2007). Vulvar pruritus and burning are hallmark symptoms in most women with VVC, and they are frequently accompanied by soreness and irritation that lead to dyspareunia and dysuria (Sobel et al., 1998; Anderson et al., 2004). Odds et al. (1988) reported that among the many signs and symptoms of VVC, pruritus and vaginal discharge showed a tendency to be correlated with the numbers of Candida in the vagina. Symptoms and signs vary considerably in intensity from mild to severely incapacitating. Vaginal discharge is not invariably present and is frequently minimal. Although it has been described as “cottage cheese-like” in character, the discharge may vary from being watery to homogenously thick. Odor, if present, is minimal and non-offensive (Anderson et al., 2004).

Physical examination should include a careful inspection of the external genitalia, vaginal sidewalls, and cervix, as well as of the discharge (Eckert, 2006). Fissures and excoriations on the external genitalia occur in approximately one in four cases of VVC but are unlikely in cases of BV and TR. Physical examination can reveal erythema and swelling of the labia and vulva, often with fissures and pustulopapular peripheral lesions. The cervix is normal, and vaginal erythema is present, together with an adherent off-white discharge (Eckert et al., 1998; Sobel, 2007). The symptoms are characteristically exacerbated during the week prior to menstruation and during pregnancy (Eckert et al., 1998).

**Epidemiology**

VVC affects approximately 20% of women annually, but it has not been well characterized epidemiologically (Geiger et al., 1995). It is not a reportable disease and is often diagnosed without mycologic confirmatory tests and treated with OTC medications; thus, its actual rate of incidence is unknown (Sobel, 1985; Sobel et al., 1998). Episodes of VVC occur mostly during childbearing years and are rare in premenarchal and postmenopausal women (Sobel et al., 1998; Geiger et al., 1995). Increasing numbers of women are self-diagnosing VVC and purchasing antifungal preparations OTC with antimycotic vaginal suppositories and creams or oral drugs (Sobel et al., 1998; Nyirjesy and Sobel, 2003). Notably, misdiagnosis is common, and studies have shown that as many as two-thirds of all OTC drugs for VVC are used by women without the disease (Ferris et al., 1996; Sobel et al., 1998; Sobel et al., 2007). Overuse of these medications can increase the risk of developing resistance to antifungal treatments (Sobel and Chaim, 1997).

A limited amount of data are available describing the incidence of VVC, and only two studies have addressed this topic, both of which relied on self-reported information from random samples of women living in the United States (Geiger et al., 1995; Foxman et al., 2000). One study reported that the frequency of the first diagnosis of VVC increased rapidly after age 17, with an estimated 54.7% of female university students reporting at least one episode of VVC by age 25 (Geiger et al., 1995). In a random digit-dialing survey of 2000 women throughout the United States, Foxman et al. (2000) found that 6.5% of women (18 years old or more) reported at least one episode of presumptive Candida vaginitis in the last 2 months. In addition, women reporting a 1-year period with four or more episodes comprised 8.0% of the sample but accounted for 37.2% of the women who reported episodes. Overall, 55.7% of women will experience at least one symptomatic episode of VVC in their lifetime (i.e., women in the survey were asked how often they thought VVC occurred) (Foxman et al., 2000). This cumulative probability appeared to be higher for African-American women compared to other ethnic groups (Geiger et al., 1995). Age appears to be an important factor in the overall incidence of VVC, such that while it is extremely rare before menarche, the annual incidence of VVC increases dramatically toward the end of the second decade of life and peaks over the next two decades (Geiger et al., 1995; Sobel et al., 1998).

**Why is C. albicans the most common Candida species?**

The predominance of C. albicans may be due to the unique ability of this form of yeast to undergo germification into a more invasive mycelial form, which is capable of invading mucous membranes and eliciting clinical symptoms. However, all pathogenic species of Candida, with the exception of C. glabrata and yeast of the genus Saccharomyces, are dimorphic (Nyirjesy and Sobel, 2003; Ledger and Witkin, 2007). Typing of vaginal C. albicans isolates failed to identify strains with tropism for the vagina (Lian et al., 2004). Similarly, there was no evidence of vaginopathic strains that showed enhanced virulence, which could explain why some women remain entirely asymptomatic despite being heavily colonized by Candida spp. (Maucra et al., 1998). Moreover, the notion of yeast pathogenicity is not without merit and could be the result of phenotypic switching and modifications in virulence properties following gene activation (Soll et al., 1989). Although clinical signs and symptoms are indistinguishable in infections caused by different Candida spp., C. glabrata, and C. parapsilosis tend to be associated with milder, or often absent, symptoms (Fidel et al., 1999; Nyirjesy et al., 2005).

**Pathogenesis**

*Candida* spp. can be present as either a commensal organism or a pathogen in the vagina, and dogma dictates that changes in the host vaginal environment are necessary for the organism to induce pathological effects (Sobel, 2007). Hyphal elements enhance colonization,
and although they represent the dominant invasive form that is capable of penetrating intact epithelial cells, only the very superficial layers are involved (Sobel, 1985). Germinated yeast that have produced pseudohyphae and/or hyphae are found most commonly in symptomatic vaginitis (Sobel, 1985). Although symptoms are not always related to the yeast load, VVC is associated with greater numbers of Candida organisms and hyphal elements (Merson-Davies et al., 1991).

Adherence
Colonization of the vagina requires yeast adherence to vaginal epithelial cells. Candida albicans adheres in significantly higher numbers to such cells than do non-C. albicans Candida species (Soll et al., 1989). It was suggested that vaginal epithelial cells play a crucial role in the defense mechanisms against VVC. In addition, it was hypothesized that following the interaction of Candida with vaginal epithelial cells, VVC is associated with signals that promote a noninflammatory polymorphonuclear neutrophil response and concomitant clinical symptoms. Moreover, resistance to VVC is associated with a lack of such signals and/or antifungal activity of vaginal epithelial cells (Barousse et al., 2005; Fidel, 2007). In another study by Fidel et al. (2004) it was proposed that the symptoms related to the presence of Candida are due to infiltration of polymorphonuclear neutrophils. However, cell-mediated immunity is considered to be a host defense mechanism against mucosal candidosis (i.e., women with RVVC do not have a defect in their systemic cell-mediated immunity, and their susceptibility is a result of an overly innate immune response) (Fidel, 2007).

On the other hand, mannose-binding lectin (MBL) is an epithelial cell-associated host protein that binds to Candida mannan, activates complement, and thus inhibits Candida growth. Reduced levels of MBL and genetic polymorphisms in the MBL gene were found in women with RVVC (Babula et al., 2003). In one study, it was noted that an MBL2 codon 54 gene polymorphism is associated with both recurrent VVC and recurrent BV (Giraldo et al., 2007).

The damage-response framework
Casadevall and Pirofski (1999, 2000) proposed the “damage-response framework” to incorporate the contributions of both the host and the microbe in microbial pathogenesis in a synthesis whereby host damage is used as a common denominator to describe the outcome of the host-microbe relationship. Host damage can result from microscopic, macroscopic, clinically apparent, or clinically unapparent events. However, the disease only becomes apparent when host damage reaches a certain threshold. Therefore, the “damage-response framework” can reconcile the apparent inconsistencies of the pathogen-centered notions of microbial pathogenesis by accounting for the host factor’s role in determining the outcome of a microbial infection. Consequently, infection is defined as the acquisition of a microbe and of all other states (i.e., colonization and disease) as an outcome of infection (Casadevall and Pirofski, 2000; Pirofski and Casadevall, 2002).

Most people who develop diseases due to so-called “non-pathogenic” or “commensal” microbes (e.g., C. albicans) have one or more of the following impairments in their immune function: (1) a breakdown of barrier immunity for microflora due to disruptions in the integument and/or mucosal niches, (2) immune suppression caused by hereditary factors or acquired diseases (e.g., HIV infection which has not been associated with an increased occurrence of VVC/RVVC unless the patients are severely immunocomprised), or (3) the use of agents to treat malignant or inflammatory diseases, and/or inhibit graft rejection after organ transplantation. Because C. albicans is part of the normal human microflora, it is clear that the discovery of C. albicans from certain sites does not represent disease. Therefore, equating infection with C. albicans with disease is not appropriate. It is most appropriate to state that humans harbor C. albicans, having acquired it soon after birth, but that diseases due to Candida spp. only occur in certain people (Pirofski and Casadevall, 2002).

Laboratory diagnosis
In addition to clinical symptoms, an accurate diagnosis of VVC currently depends on the demonstration of Candida sp. or spp. in vaginal swabs using vaginal pH measurement, microscopic examination, and/or fungal culture (Abbott, 1995; Sobel et al., 1998; Sobel, 2007). A positive Gram stain, the absence of a watery discharge, and patient self-diagnosis of an “another yeast infection” have been identified as the best predictors of a positive culture for patients with VVC (Abbott, 1995).

Vaginal pH
A major factor influencing the microbial composition of the vagina is the vaginal pH (Ledger and Witkin, 2007). A simple and inexpensive test to evaluate the pH has been greatly underused in cases of suspected VVC (Sobel et al., 1998). Vaginal pH should be measured by touching a cotton-tipped swab to the sidewall of the vagina midway between the introitus and the cervix, and then touching the swab to commercially available pH paper. The pH should not be tested by sampling the vaginal pool in the posterior fornix because the pH of this region may be elevated by the presence of cervical mucus (Eckert, 2006). The vaginal pH is normally acidic (4.0–4.5) in VVC, whereas a pH value in excess of 4.7 usually indicates BV, TR, or a mixed infection (Sobel et al., 1998; Nyirjesy and Sobel, 2003; CDC, 2006; Sobel, 2007). VVC may complicate BV and TR, as well as cervicitis and upper genital infections. The true frequency of mixed infections is unknown but is estimated to be 10% in cases of vaginitis and may be considerably higher in patients seen in sexually transmitted disease clinics (Sobel et al., 1998).
Clinical samples
For the purpose of an accurate diagnosis, vaginal samples are obtained from the lateral wall using a cotton-tipped swab and are inoculated into 1 ml of Sabouraud 4% glucose broth for transport to the laboratory. In one study, intraoital and vaginal lavage samples were compared to posterior vaginal fornix samples to detect the rate of *Candida* yeasts in RVVC cases; no differences were detected (Novikova et al., 2002). In another study, it was proposed that extragenital sites (e.g., the mouth, the perianal skin, and the rectum) might be reservoirs for recolonization of the genital tract in symptomatic women with RVVC. The authors concluded that the perianal skin is a possible reservoir for recolonization of the vagina, and investigations of extragenital sites for *Candida* are strongly recommended (Mårdh et al., 2003).

Microscopic examination
None of the clinical manifestations of VVC is pathognomonic; therefore, clinical diagnosis must always be confirmed by laboratory methods. Microscopy is the mainstay in the diagnosis of VVC; however, studies have shown that, microscopy has a sensitivity of 50% (range 40–60%) in diagnosing this condition and overlooks a substantial percentage of cases in women with VVC (Bergmann et al., 1984; Nyirjesy et al., 1995). VVC is generally diagnosed via positive wet-mount and/or Gram-stain preparations that demonstrate budding yeasts, pseudohyphae, and/or hyphal forms (Bergmann et al., 1984; CDC, 2006). Approximately 50% of patients have a positive result by microscopy of wet-mount or saline preparations, in which yeast cells and hyphal elements can be visualized (CDC, 2006). Microscopy is also used to exclude the presence of so-called “clue cells” that are indicative of BV, as well as motile trichomonads (Sobel et al., 1998; Sobel, 2007; Achkar and Fries, 2010). The presence of pseudohyphae is thought to be associated with a higher likelihood of symptomatic VVC. However, a negative microscopy result together with a small number of yeast cultured is considered to indicate *Candida* colonization (< 10 colonies), rather than symptomatic infection (Ledger and Witkin, 2007).

A 10% potassium hydroxide (KOH) preparation is more sensitive (65–85%) than a saline (~40%) preparation in identifying fungal elements, e.g., blastospores and hyphae (Sobel, 1999; Sobel, 2007; Achkar and Fries, 2010). Because it lyse vaginal epithelial cells, the addition of a 10% KOH solution to patient specimens may facilitate the visualization of fungal elements (Nyirjesy and Sobel, 2003). However, in one study, similar accuracy rates were noted with 10% KOH (36%) and saline (38%) based on microscopic examinations (Abbott, 1995). Additionally, Bergmann et al. (1984) support the assertion that the KOH preparation is only occasionally useful, as it has demonstrated poor sensitivity (19%). It should be noted that *C. glabrata*, the second leading cause of VVC, produces only blastospores, which may be difficult to identify with in-office procedures (Spinillo et al., 1997; Nyirjesy and Sobel, 2003).

Gram-staining provides the most accurate and rapid microscopic predictor (n=15) of a positive culture (n=23), recognised as the gold standard. In addition, Gram-staining had 65% sensitivity and negative predictive value (NPV), as well as a 100% specificity and positive predictive value (PPV) (Abbott, 1995). On the other hand, the Gram-stain together with the wet preparation is considered the gold standard for rapid diagnosis of VVC (CDC, 2006).

However, many clinicians are unable or unwilling to measure the vaginal pH and perform microscopy (Sobel et al., 1998; Sobel, 2007). Therefore, most women are routinely diagnosed without appropriate mycological information, and in as many as half of the cases studied, the women may be uninfected or may have another condition (Sobel et al., 1998; Sobel, 2007). Several studies have noted that up to 50% of culture-positive patients demonstrate negative microscopy results (Sobel, 1985; Eckert et al., 1998). Although routine cultures are unnecessary when microscopy results are positive, vaginal culture is more sensitive and should be performed for symptomatic women with negative microscopy results and a normal pH (Bergmann et al., 1984; Eckert et al., 1998; CDC, 2006). Fungal cultures should be performed routinely: (1) in cases of RVVC, (2) in HIV-positive patients with suspected VVC (Spinillo et al., 1997), and (3) to obtain surveillance cultures and monitor changing trends in the microbiology of VVC cases (Sobel et al., 1998). To ensure appropriate and timely therapy and an awareness of new emerging pathogens, it is imperative that the yeast present be identified accurately to the species level (Pincus et al., 2007). Identification methods and up-to-date algorithms have been discussed extensively in two excellent reviews (Freydière et al., 2001; Pincus et al., 2007).

Additionally, the Pap-smear is an insensitive but highly specific method of diagnosis, yielding positive results in only about 25% of patients with culture-positive symptomatic VVC (Donders et al., 1992; Sobel, 2007). The reason for this insensitivity is that Pap-smears are used for the purpose of screening for cervical dysplasia and are taken from the squamocolumnar junction in the cervix, which is a suboptimal site for VVC diagnostics. Thus, this test is not designed for detecting VVC or other genital infections (Donders, 2007).

Fungal culture
**Sabouraud dextrose agar**
The gold standard for diagnosis is still the growth of the infecting organism in fungal culture on Sabouraud dextrose agar (SDA). This selective medium was devised by a French dermatologist, Raymond Sabouraud (1864–1938), to cultivate dermatophytes in 1894 and is the preferred medium in most microbiology laboratories. SDA is a general-purpose medium that supports the growth of most pathogenic fungi, including *Candida* and other yeast species. However, SDA is not a differential medium,
and colonies of different yeast species grown on this agar cannot be easily distinguished from one another (Odds, 1991; Odds et al., 1994).

The inclusion of peptones and dextrose in SDA encourages the growth of yeast. On SDA, however, the isolation and identification processes used in many standard phenotypic assays can take up to 2–4 d (Pincus et al., 2007). The use of differential media, such as chromogenic agars, can shorten the time to diagnosis of yeast infections by differentiating common Candida species based on colony color (Odds et al., 1994). These ready-to-use chromogenic media are simpler and allow culture, isolation, and identification on the same Petri dish (Freydiére et al., 2001; Pincus et al., 2007). However, chromogenic media are more expensive than traditional media, such as SDA (Pincus et al., 2007).

Any mycological culture method will result in occasional false-negatives. In symptomatic women with a normal vaginal pH, if both microscopy and culturing are performed by a competent laboratory diagnostician, then the failure rate will almost certainly be < 10%. However, in one study, in patients with a history of RVVC who presented with symptoms and signs of a new attack, half of the women proved to be culture-negative for Candida (Novikova et al., 2002). In addition, Nyirjesy et al. (1995) noted that 75.3% of patients with chronic vaginal symptoms had a negative fungal culture on SDA. Interestingly, Anson and Allen (1997) detected yeast species only in 14.1% of patients with VVC using CHROMagar Candida (CAC, Becton Dickinson) and malt extract agar. In a study of 61 women with VVC based on clinical examination and microscopy, Ledger et al. (2000) described that 49% had a negative fungal culture and PCR result. Therefore, the presence of Candida spp. cannot always be confirmed.

Chromogenic media

Clinical microbiology laboratories face an important challenge in selecting a system for yeast identification that is accurate, cost-effective, easily interpreted, and reasonably rapid (Freydiére et al., 2001). Selective and differential media for the primary isolation of Candida species were reported over 50 years ago (Nickerson, 1953; Pagano et al., 1957–58). During the ensuing period, chromogenic media have been formulated that are globally marketed and widely discussed in the literature. Due to the differences in the morphologies and colors of yeast colonies, these media facilitate the detection of common Candida species, e.g., C. albicans and C. tropicalis, according to the package inserts (Odds et al., 1994; Pfaller et al., 1996; Willinger et al., 2001; Novikova et al., 2002; Ilkit et al., 2007; Pincus et al., 2007; Ozcan et al., 2010; Guzel et al., 2011). These differential media integrate the chromogenic detection of β-N-acetylhexosaminidase and either phosphatase or β-glucosidase as a second enzymatic activity (Pincus et al., 2007). The species identified on chromogenic agars correspond well to those obtained by fermentation and assimilation tests (Pfaller et al., 1996; Pincus et al., 2007). Recently, we reported that chromogenic agars could be integrated into the laboratory workflow as primary isolation media (Ozcan et al., 2010). The prominent advantages of these media include the following: (1) improved recovery rates of yeasts, (2) rapid identification of pathogens, and (3) detection of polyfungal populations in clinical specimens (Freydiére et al., 2001; Pincus et al., 2007). The list of the available chromogenic media is presented in Table 1.

In an earlier study, no difference in the isolation rate was detected using SDA, Nickerson’s medium (Ortho Pharmaceuticals), and Microstix-Candida medium (Bayer Healthcare) for fungal culture (Bergmann et al., 1984). Recently, we reported the detection of yeast fungi in 40.1% of VVC cases using CAC and Candida Chromogenic Agar (Ozcan et al., 2010). More recently, we detected yeast in a total of 43.2% of the patients in a group that consisted of acute and recurrent cases of VVC and healthy controls, with detection rates of 49.2%, 44.2%, and 34% in each subgroup, respectively (Guzel et al., 2011). Moreover, we observed that SDA was less sensitive (92.2%) for the detection of Candida spp. in comparison to CAC (95.1%) and chromID Candida agar (96.6%) (CAN2, bioMérieux), particularly in patients with RVVC. In addition, 7.8% of the culture-positive patients would have tested culture-negative if SDA had been the only medium used (P = 0.17). Therefore, the higher recovery rates observed may be due to the use of three different study media (Guzel et al., 2011). The recovery rate for C. albicans was similar for SDA, CAC, and CAN2, and we also observed no differences with non-C. albicans Candida species (Guzel et al., 2011). In one investigation, it was noted that CAC supported the growth of Candida spp. as well as SDA (Pfaller et al., 1996). These authors reported that CAC was more sensitive than SDA for the isolation of C. tropicalis and less sensitive for C. parapsilosis; however, the authors also noted that the reasons underlying these differences were unknown, even though each yeast strain has different optimal growth conditions in terms of substrate and temperature (Pfaller et al., 1996).

Notably, the use of chromogenic media improves the rapid and accurate diagnosis of C. albicans with a sensitivity and specificity of 94.1% and 100%, respectively, and improves the detection of polyfungal populations in vaginal specimens (Ozcan et al., 2010; Guzel et al., 2011). In addition, the correct identification of Candida species greatly influences the recommended course of therapy.

Table 1. Commercial chromogenic media for recovery and rapid identification of Candida spp.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brilliance Candida agar</td>
<td>Oxoid</td>
</tr>
<tr>
<td>Candida Diagnostic agar</td>
<td>PPR Diagnostics</td>
</tr>
<tr>
<td>CandiSelect 4</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>Chromalbicicans agar</td>
<td>Biolife</td>
</tr>
<tr>
<td>ChromID Candida agar</td>
<td>BioMérieux</td>
</tr>
<tr>
<td>CHROMagar Candida</td>
<td>CHROMagar Microbiology</td>
</tr>
<tr>
<td>BBL-CHROMagar Candida</td>
<td>Becton-Dickinson</td>
</tr>
<tr>
<td>HiChrome Candida agar</td>
<td>Himedia</td>
</tr>
</tbody>
</table>
Although *C. albicans* is still the most commonly detected pathogenic yeast fungus, fluconazole-resistant *C. albicans* isolates have also been reported (Sobel and Vazquez, 1996; Dorrell and Edwards, 2002; Sobel et al., 2003).

**Polyfungal colonization**

It is not uncommon for vaginal samples to yield multiple *Candida* species (Bouchara et al., 1996; Ozcan et al., 2010; Guzel et al., 2011). Several studies have reported that the percentage of polyfungal populations in vaginal samples ranges from 1.3% to 14.1% (Anson and Allen, 1997; Richter et al., 2005; Hospenthal et al., 2006; Ozcan et al., 2010; Guzel et al., 2011). Notably, polyfungal populations may not be detected on SDA, which is routinely used to culture clinical samples (Ozcan et al., 2010; Guzel et al., 2011). When azole-resistant non-*C. albicans* *Candida* strains are not accurately detected, prompt and appropriate therapy may be delayed (Anson and Allen, 1997). We recently observed that polyfungal populations were detected and similarly distributed among acute and recurrent VVC cases and in patients without VVC (*P* = 0.5).

Polyfungal populations in vaginal samples were detected at a higher rate using CAN2 compared to CAC (96.6% vs. 86.2%), but this difference was not significant (*P* = 0.35) (Guzel et al., 2011). When multiple species were present, one of the isolates was always *C. albicans*. This result was not surprising because *C. albicans* is the most prevalent yeast in clinical specimens (Bouchara et al., 1996; Pfaller et al., 1996; Willinger et al., 2001; Richter et al., 2005; Ozcan et al., 2010; Guzel et al., 2011). The combination of *C. albicans* and *C. glabrata* has been previously observed to occur in 47.6% (Ozcan et al., 2010), 77.8% (Richter et al., 2005), and 86.2% (Guzel et al., 2011) of polyfungal populations in vaginal samples. Therefore, cases of *C. glabrata* may be misdiagnosed or underreported when SDA is used alone without the benefit of chromogenic media. Consequently, at least one chromogenic medium should be used for laboratory diagnoses of VVC (Guzel et al., 2011).

**Antigen tests**

Several rapid diagnostic tests have been developed over the past three decades in an attempt to accelerate the diagnosis of VVC (Hopwood et al., 1985; Reed and Pierson, 1992). The presence of rapid, simple, and inexpensive diagnostic tests may solve the problems of both overdiagnosis and underdiagnosis of VVC (Sobel, 2007). It was concluded that antigen tests, which can be administered either by a health care professional or by the patient herself, do not require sophisticated equipment, are simple to use and are easy to interpret (Chatwani et al., 2007; Dan et al., 2010). This type of test can be used to identify vaginal *Candida* infections in symptomatic patients during office visits, and can thereby lead to prompt and accurate therapy, save time, improve compliance, and shorten the period of suffering. In addition, costs will be decreased by limiting the use of unnecessary drugs by physicians and diminishing patient self-medication (Dan et al., 2010).

The detection of *C. albicans* antigens in vaginal discharge has been reported as a useful alternative method for the rapid diagnosis of VVC. Hopwood et al. (1985) investigated a total of 87 vaginal specimens and reported that the latex particle agglutination (LPA) test had 80% (24/30) sensitivity, 100% (57/57) specificity, 91% (57/63) NPV, 100% PPV (24/24), and a 93% accuracy rate (the number of true positives (*n* = 24) + the number of true negatives (*n* = 57)/total (*n* = 87)) compared with the gold standard (microscopy and fungal culture). The authors suggested that some of the false-negative reactions detected using their LPA test could have been due to infections caused by *C. albicans* serotype B or non-*C. albicans* *Candida* species because the polyclonal antibodies used to sensitize the latex particles were raised against *C. albicans* serotype A. Abbott (1995) reported poor performance for the rapid LPA test (Candidasure, Medical Technology Corporation) in diagnosing 71 cases of VVC [i.e., 81% sensitivity, 59% specificity, 48% PPV, and 88% NPV compared to positive *Candida* cultures (*n* = 23)]. However, these data were not presented clearly to clarify the prevalence of these numbers. In addition, Pike et al. (1991) used an ELISA to detect *Candida* mannan in vaginal washings from 40 women with *C. albicans* vaginitis and found a significant association between mannan levels and clinical signs. However, the authors did not report the sensitivity and specificity of this test. Interestingly, Tan et al. (2003) assessed an ELISA-based test (SysCan3, Rockeby Biomed Ltd, Perth, Australia) using sera from 50 patients (47 with VVC and 3 without VVC). This test had a sensitivity of 77.5% (31/40) and a specificity of 90% (9/10) compared with the positive cultures (*n* = 40). However, no further studies have validated the potential value of serological tests for VVC or RVVC.

New and rapid immunochromatography tests (ICTs) have been developed for the detection of vaginal *Candida* species (Chatwani et al., 2007; Matsui et al., 2009; Marot-Leblond et al., 2009; Dan et al., 2010). ICT is based on the capture of *Candida* mannan from vaginal secretions using the IgM mouse MAb 5B2. This MAb recognizes mannan from vaginal secretions using (Savvycheck, Savvy Diagnostics, Ashdod, Israel) to compare a rapid yeast test with culture tests and reported a sensitivity and specificity of 77.4% and 76.9%, respectively. Dan et al. (2010) examined a polyclonal antibody-based ICT for the rapid detection of VVC and reported a sensitivity of 79%, a specificity of 96%, and 87% NPV; these results were compared with those obtained for fungal culture.

Marot-Leblond et al. (2009) developed a ICT test (CandiVagi assay, SR2B, Avrille, France) that had a significantly higher sensitivity (96.6%) than microscopic examination including wet mount and/or Gram-stain
preparations (61.6%, \( P > 0.001 \)) and a higher specificity (98.6%) than fungal culture (82%, \( P > 0.001 \)). Furthermore, the authors reported a greater sensitivity using ICT than with the LPA tests used to detect *Candida* antigens in vaginal secretions (96.6% vs. 66–80%) (Hopwood et al., 1985; Reed and Pierson, 1992). The reason for the association of a positive ICT reaction with infection rather than colonization remains unclear but may be related to the characteristics of the antigens detected by MAb 5B2 (Maror-Leblond et al., 2009). In addition, Matsui et al. (2009) developed an ICT that could detect as few as 10^3 cfu/ml of *Candida* spp. in approximately 30 min without using specialized equipment or highly trained technicians. A total of 200 clinical vaginal swabs were examined, and overall, the sensitivity, specificity, PPV, and NPV of this method were found to be 80.3% (49/61), 99.3% (139/139), 98.0% (49/50), and 92.0% (138/150), respectively, compared with those obtained by the culture method. As a result, the aforementioned studies attributed high value to this diagnostic method. However, the majority of these investigations were never validated by a second study, and none has stood the test of time and is currently in clinical use.

**Typing of Candida strains**

In the past two decades, a variety of molecular techniques have emerged that provide a high degree of reliability in epidemiological tracking of various strains of pathogenic yeasts, e.g., RAPD (Daniels et al., 2001). The genetic relatedness of *Candida* strains in women with RVVC has been studied and three scenarios have been put forth, as follows: (1) VVC strain maintenance without genetic variation, (2) strain maintenance with minor genetic variation, and (3) strain replacement by a different strain (Lockhart et al., 1996).

Strain maintenance over long periods appears to be common, but studies that have employed very sensitive genotyping methods have revealed the possibility of microevolution among colonizing strains (Lockhart et al., 1995; Lockhart et al., 1996; Chong et al., 2003). In one study, the same strain was found to be responsible for sequential infections in all 18 cases of RVVC investigated, which suggests that the predominant scenario is strain maintenance with minor genetic variations, a process that the authors termed “substrain shuffling” (Lockhart et al., 1996). Recently, Chong et al. (2003) confirmed strain maintenance with minor microevolution in the majority of women with recurrent episodes of VVC but also detected evidence of strain replacement by an unrelated strain or species [14.3% (4/28)]. In a longitudinal-DNA typing study, it was noted that eight of 10 patients with RVVC maintained a single strain of *C. albicans* through sequential infectious episodes; however, in two patients, the strain had undergone replacement by another strain (Vazquez et al., 1994). Stein et al. (1991) used an RFLP method to determine the presence of different strains in the vagina and rectum and concluded that recurrent vaginitis is usually due to a relapse with the same vaginal strain, rather than reinfection from the rectum. Daniels et al. (2001) collected isolates of *C. albicans* from women who were followed longitudinally through their pregnancies, and identified six pairs of cultures from women who were colonized without exhibiting symptoms and who later became symptomatic after an average of 14 weeks. Analysis of these pairs by RAPD revealed that five of the six women had symptoms that were apparently due to the same yeast strain that was found initially as a commensal strain. The authors suggested that the changing relationship between the host and the microorganism during the progression of pregnancy was probably more important in the development of symptoms than was replacement, or even genetic changes in the commensal organism.

Wenjin and Yifu (2006) genotyped *C. glabrata* isolates from 50 pregnant women and described 17 RAPD profiles. The number of genotypic patterns obtained during the last 2 trimesters (10 and 11 genotypes, respectively) was greater than from the first trimester (7 genotypes), especially for symptomatic patients. These results showed that the RAPD patterns of *C. glabrata* during pregnancy were highly polymorphic. Singh et al. (2003) analyzed 26 vaginal *C. krusei* isolates from 12 patients with VVC, and six different *C. krusei* genotypes were identified. Therefore, molecular data are essential to demonstrate the epidemiological relatedness of *Candida* spp.

**Source of infection**

The information presented in this section has been extensively discussed in several reviews by Dr. Sobel (1985, 1987), including sections referencing the transmission of *Candida* yeast. The types of transmission are as follows: (1) intestinal reservoir, (2) sexual transmission (penile-vaginal), and (3) vaginal relapse. Although the gut and sexual transmission are assumed to be the main sources of vaginal colonization, published studies report conflicting data (Mendling et al., 2000; Lisboa et al., 2011). For example, Mendling et al. (2000) reported that identical *C. albicans* strains were obtained from the vaginas of 4 of 21 (19%) patients with RVVC and from the semen of their partners by PCR fingerprinting. More recently, however, Lisboa et al. (2011) discovered that women suffering from RVVC were more likely to have an asymptomatic *Candida*-negative sexual partner than non-recurrent VVC women. The authors noted that the male genitalia do not represent a relevant *Candida* reservoir for RVVC compared to the female endogenous reservoir. However, the anal sex experience of these individuals was not recorded, and anal samples were not processed.

Vaginal relapse is thought to be the most important source of infection; the recurrence of symptomatic vaginal infection may result from a failure of conventional therapy to completely eradicate *Candida* from the vaginal lumen and potentially from the superficial vaginal mucosal tissue (Sobel, 1985; Stein et al., 1991; Vazquez et al., 1994; Lockhart et al., 1995; Lockhart et al., 1996; Chong et al., 2003).
who has the attitude and the substance of a genius and gynecologist emeritus, Professor Nihat Arıdogan, this study is dedicated to an outstanding obstetrician

**Acknowledgment**

This study is dedicated to an outstanding obstetrician and gynecologist emeritus, Professor Nihat Arıdogan, who has the attitude and the substance of a genius and continually and convincingly conveyed a spirit of adventure in regard to research and an excitement in teaching for over 25 years in Çukurova University, Turkey.

**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

**References**


Hopwood V, Evans EGV, Carney JA. (1985). Rapid diagnosis of vaginal
of Albicans ID2 and Biggy agar for the isolation and direct
Candida stellatoidea? Disappearing from the the vaginal mucosa? J
Microbiol, 28, 600–1.
Ledger WJ, Polaneczky MM, Yih MC, Jeremias J, Tolbert V, Witkin SS.
Pract, 9, 66–9.
associated with different conditions of vulvovaginal candidiasis.
Mycoses, 47, 495–502.
clonal in origin but undergo microevolution through Cl fragment
reorganization as demonstrated by DNA fingerprinting and Cl
scenario for recurrent Candida vaginitis is strain maintenance
with "substrain shuffling"; demonstration by sequential DNA
fingerprinting with probes Ca3, Cl, and CARE2. J Clin Microbiol,
34, 767–77.
diagnosis of vaginitis compared with a DNA probe laboratory
Márdh P-A, Rodrigues A, Genc M, Novikova N, Martínez-de-Oliveira
J, Guaschino J. (2002). Facts and myths on recurrent vulvovaginal
candidiasis: a review of epidemiology, clinical manifestations,
diagnosis, pathogenesis, and therapy. Int J STD AIDS, 13,
522–39.
extragenital sites by Candida in women with recurrent vulvovaginal
Marot-Leblond A, Nail-Billaud S, Pilon F, Beucher B, Poulin D,
by use of a new rapid immunochromatography. J Clin Microbiol,
47, 3821–5.
Martinez FR, Franceschini SA, Patta MC, Quintana SM, Nunes AC,
Moreira IL, Anukam KC, Reid G, De Martinis EC. (2008). Analysis
of vaginal lactobacilli from healthy and infected Brazilian women.
Appl Environ Microbiol, 74, 4539–42.
Matsui H, Hanaki H, Takahashi K, Yokoyama A, Nakae T, Sunakawa
by newly developed immunochromatography test. Clin Vacc
McClelland RS, Richardson BA, Hassan WM, Graham SM, Kiarie J,
Baeten JM, Mandaliya K, Jiao W, Ndinya-Achola JO, Holmes
KK. (2009). Prospective study of vaginal bacterial flora and
other risk factors for vulvovaginal candidiasis. J Infect Dis, 199,
1883–90.
Mending W, Pinto de Andrade M, Gutschmidt I, Cenjetnig R, Presber
different locations of women suffering from vaginal candidiasis,
Merson-Davies LA, Odds FC, Malet R, Young S, Riley V, Schober P,
Fisk PG. (1991). Quantification of Candida albicans morphology in
Nickerson WJ. (1953). Reduction of inorganic substances by yeasts.
lavage samples and cultures on chromogenic agar? Infect Dis Obstet Gynecol, 10, 89–92.


