



Overview of Vaginitis: Office-Based DNA Testing

Haywood L. Brown, MD

Given that patients are likely to assume that any vaginal pruritus and discharge are due to candidiasis, the high incidence of self-misdiagnosis and consequent failure of over-the-counter (OTC) therapy are hardly surprising. Patients should be reassured that effective treatments are available provided they rely on the physician for accurate diagnosis.

Complaints of vaginal discharge, vaginal odor, and/or vulvar itching account for more than 10 million physician office visits and 10% of all outpatient visits in the United States annually.¹ Vaginitis-related health care costs are estimated at more than \$500 million per year, not including efforts at self-treatment.¹ Failure of symptoms to resolve with OTC preparations typically prompt the woman to contact a physician.

Landers et al² noted that treatment should not be directed by symptoms alone, and that diagnostic

uncertainty or treatment failures should prompt specific laboratory testing to ensure appropriate therapy. More than 90% of infectious vaginitis is caused by bacterial vaginosis (BV), fungal infections (candidiasis), and parasitic infections (trichomoniasis). Bacterial vaginosis is the most common of the three, and has the most stringent criteria for accurate diagnosis.

NORMAL VAGINAL ECOSYSTEM

Disruption in the normal vaginal ecosystem changes the microflora of the healthy vagina, altering vaginal pH and predisposing to lower-tract infections such as vaginitis. A balanced vaginal ecosystem depends on *Lactobacillus* spp, which are essential for maintaining the vaginal pH at < 4.5 and assisting host defenses to inhibit overgrowth of other pathogenic bacteria (eg, obligate anaerobes) that can lead to infection. In the healthy vagina, *Lactobacillus* spp are present at >10⁶ CFU/mL of vaginal fluid. Pathogenic bacteria are conducive to an environment that decreases lactobacilli to < 10³ bacteria/mL of vaginal fluid.

CANDIDA VAGINITIS

Candida infections account for approximately 33% of all vaginitis cases.¹ *Candida* vaginitis is most common in premenopausal women.² As estrogen levels are a contributing factor, reproductive-aged women (especially those using oral contraceptives) and postmenopausal women using estrogen therapy are at greatest risk. *Candida* vaginal colonization is thought to occur in 10% to 55% of healthy, asymptomatic women.³ Infection rates are estimated at a single episode in 75% of reproductive-aged women,⁴ more than one episode in 45%,⁵ recurrent infection in 5%, and persistent/chronic

Haywood L. Brown, MD, is professor and chair, Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC.

Dr Brown reports no conflict of interest.

Supported by BD Diagnostics and produced by *The Female Patient*.

Overview of Vaginitis

FEATURES OF VAGINITIS*

	Candida	Bacterial Vaginosis	Trichomonas
Symptoms	Pruritus White discharge Dyspareunia	Gray discharge Odor	Purulent discharge Odor Dyspareunia
Signs	Vulvar erythema	Discharge	Vulvovaginal erythema
Vaginal pH	4.0-4.5	> 4.5	4.0-6.0
Amine test	Negative	Positive	Variable
Wet mount	Pseudohyphae WBCs	Clue cells	WBCs Trichomonads

*May have mixed infection.
WBCs = white blood cells.

infection in 1%. There is a relationship to the occurrence of yeast infections and the frequency of sexual activity⁶ and the use of injectable progestin contraception.⁷ Approximately 70% of young, sexually active women will experience vaginal colonization with yeast at some time during a 1-year period.⁷

Candida albicans is responsible for 90% to 92% of *Candida* vulvovaginitis.^{2,8} However, *Candida glabrata* (*Torulopsis glabrata*) and other *Candida* spp are appearing with increasing frequency. Visualization of the yeast buds (pseudohyphae) is important for clinical diagnosis with microscopic saline/potassium hydroxide (KOH) wet-mount examination.

TRICHOMONAS VAGINITIS

Trichomoniasis accounts for 15% to 20% of all vaginitis, but is probably underdiagnosed due to lack of confirmatory testing.⁹ *Trichomonas* is most prevalent in women aged 20 to 45 years, and approximately 25% to 50% of women with trichomoniasis are asymptomatic. Based on World Health Organization estimates, there are approximately 5 million new cases in the United States and 180 million new cases world-wide annually.¹⁰ The protozoan responsible for vaginal infection is *Trichomonas vaginalis*, which is one of the five known Trichomonadidae species.¹¹ Growth is facilitated by a pH > 5 and an anaerobic environment. In vitro studies indicate that bacteria such as *Micrococcus*, *Staphylococcus aureus*, and *Enterococcus faecalis* appear to act synergistically with *T vaginalis*, whereas *Proteus vulgaris* and *Pseudomonas aeruginosa* appear to be antagonistic.¹² Organisms such as *Escherichia coli* and *Mycoplasma hominis* can attach to the protozoan and travel into the upper genital tract, leading to postoperative morbidity from gynecologic surgery in women colonized preoperatively with *T vaginalis*.¹³

One of the strongest risk factors for trichomoniasis is a history of sexually transmitted infections (STIs). The presence of *T vaginalis* also increases susceptibility

to BV,¹⁴ and coinfection with both entities may also promote acquisition of human immunodeficiency virus (HIV).¹⁴ Women with trichomoniasis during pregnancy have a higher risk for adverse outcomes, including premature rupture of membranes, preterm delivery, and low birthweight.¹⁵

BACTERIAL VAGINOSIS/VAGINITIS

Bacterial vaginosis is the most common of the vaginal infections, accounting for 15% to 50% of vaginitis/vaginosis.^{16,17} This is

a complex disorder that has been associated with an increased risk of other infectious conditions of the genital tract and premature delivery in pregnancy. There is a significant association between BV and acquisition of STIs, including pelvic inflammatory disease (PID),¹⁸ HIV,¹⁹ and obstetric/postoperative infections. The microflora of BV consists of facultative and obligate anaerobic bacteria that overcome the normally dominant lactobacilli in the vagina. *Gardnerella vaginalis* is the major cause of infection in BV,²⁰ leading to a pH shift to > 5 and permitting the growth of various obligate anaerobic bacteria.

DIAGNOSIS

The predominant symptoms of vaginitis are vaginal discharge, vaginal odor, vulvar and/or vaginal irritation, and pruritus. Characteristics of the healthy vaginal ecosystem include:

- A white to slate-gray discharge that is liquid or pasty
- Absence of vaginal odor
- Vaginal pH of 3.8 to 4.5
- Estrogenized squamous epithelial cells
- White blood cell (WBC) count < 4/hpf
- Abundant lactobacilli

Traditional office diagnosis depends on characteristics of the vaginal discharge, saline/KOH wet-mount findings, and vaginal pH.

Candidiasis

In candidiasis, the patient presents with vulvovaginal pruritus. The typical itching/burning sensation develops within 24 to 72 hours after intercourse. The partner may also complain of transient genital discomfort. Although vulvovaginal burning and itching are often considered pathognomonic for yeast infection, these symptoms are not specific for yeast, and can lead to inappropriate treatment. In a review by Ferris et al,²¹ only 33.7% of women evaluated who had self-diagnosed and purchased OTC treatments for vulvovaginal candidiasis actually had candidiasis.

TREATMENT OF UNCOMPLICATED VAGINITIS

Vaginitis	Treatment Options
Candida	Butoconazole, 2% intravaginal cream qd for 3 d Clotrimazole, 1% intravaginal cream qd for 7-14 d Clotrimazole, 100-mg vaginal tablet qd for 7 d Clotrimazole, 100-mg vaginal tablets bid for 3 d Clotrimazole, 500-mg vaginal tablet in single dose Fluconazole, 150 mg po in single dose Miconazole, 2% intravaginal cream qd for 7 d Miconazole, 100-mg vaginal suppository qd for 7 d Miconazole, 200-mg vaginal suppository qd for 3 d Terconazole, 0.4% intravaginal cream qd for 7 d Terconazole, 0.8% intravaginal cream for qd 3 d Terconazole, 80-mg vaginal suppository qd for 3 d Tioconazole, 6.5% intravaginal ointment in a single dose
Trichomonas*	Metronidazole, 2 g po in a single dose Metronidazole, 500 mg po bid for 7 d Tinidazole, 2 g po in a single dose
Bacterial Vaginosis	Clindamycin, 2% intravaginal cream hs for 7 d Metronidazole, 500 mg po bid for 7 d Metronidazole, 0.75% intravaginal gel qd for 5 d

*Treat male partners.
qd = once daily; bid = twice daily; po = orally; hs = at bedtime.

On clinical examination, the patient may exhibit no obvious genital changes to mild to severe erythema of the vulva and introitus. The consistency of the discharge is typically white and viscous (“cottage cheese”-like), but may range from liquid to pasty. The vaginal epithelium may be erythematous, with the thick discharge adherent to the vagina walls and cervix.

Microscopic saline wet-mount examination may reveal classic pseudohyphae. Mixing the specimen with 10% KOH will dissolve other bacterial and epithelial cells and cellular debris, facilitating observation of the yeast.

Trichomoniasis

Trichomonas vaginitis generally presents with a malodorous vaginal discharge. The patient may also describe vulvar irritation or pruritus, soreness, and dyspareunia. Vaginal examination demonstrates a liquid, dirty-gray or greenish, frothy, malodorous discharge. Cervical petechial hemorrhaging occurs in 25% of cases (ie, “strawberry” cervix). A sample of the vaginal discharge will have a pH > 5. Microscopic saline wet-mount examination establishes the diagnosis in 75% of cases by visualization of trichomonads.

If the typical purulent discharge is present, the pH is > 5, and there are numerous WBCs but no clue cells or trichomonads, trichomoniasis is still likely. Additional diagnostic studies may include culture for *T vaginalis* using Diamonds media or DNA hybridization (Affirm VPIII Microbial Identification Test, Becton Dickinson

Diagnostic Systems, Sparks, Md.). Culturing improves diagnostic sensitivity and specificity compared with wet-mount analysis, but is time-consuming.²

A rapid assay has been devised for detection of *Trichomonas* using immunochromatographic capillary-flow dipstick technology to detect antigens. This test performs as well as or better than wet-mount analysis, and does not require microscopy.²²

Bacterial Vaginosis

The clinical diagnosis of BV requires the presence of three of four criteria²³: a thin, homogeneous, dusty-colored discharge; vaginal pH > 4.5; a positive “whiff” test (release of amine odor with the addition of 10% KOH); and demonstration of clue cells on microscopic wet-mount examination. Gram staining has been considered the “gold standard” for diagnosis of BV, but is rarely employed in clinical

practice today. Nugent et al²⁴ proposed a scoring system based on Gram stain and visual recognition of bacterial morphotypes. Demonstration of clue cells on wet mount is variable, and depends on the adequacy of the specimen. In addition, recent intercourse, douching, menstruation, or cervical mucus can have an impact on the vaginal pH.²⁵

Interpretation

A combination of clinical signs and symptoms with office-based tests and microscopy may prove inadequate for diagnosis, even though this is superior to a solely symptom-based approach. Landers et al² examined 598 women with vaginal discharge using wet-mount analysis with saline and KOH, pH testing, Gram stain, *T vaginalis* culture with Diamonds media, and yeast culture with Sabouraud’s agar. Bacterial vaginosis was diagnosed in 46% of subjects, candidiasis in 29%, and trichomoniasis in 12%.² Coinfection with BV and *T vaginalis* was seen in 21%, and no infection was detected in 12%.² Both *T vaginalis* and the microorganisms comprising BV are anaerobic and produce similar signs, creating the potential for misdiagnosis.² Clinical diagnosis based on both symptoms and office-based testing improved diagnostic accuracy for BV and trichomoniasis, but not so much for candidiasis.² Even in the presence of pruritus, the likelihood of detecting yeast was only 22%, and most women with positive cultures were not diagnosed clinically.²

Overview of Vaginitis

New diagnostic methods such as DNA hybridization have improved diagnostic reliability. Boggess et al²⁶ compared DNA hybridization with Gram stain for detection of BV (*G vaginalis*, *Prevotella bivia*, *Bacteroides ureolyticus*, *Mobiluncus curtisii*) in asymptomatic pregnant women. Vaginal pathogens were detected by DNA hybridization in 43% of women, versus 26.1% detected by Gram stain. This test identifies *Candida* spp, *G vaginalis*, and *T vaginalis* nucleic acid in vaginal fluid specimens, and has been tested in several populations.^{27,28}

One study measured the performance of the Ambient Temperature Transport System (Affirm VPIII ATTS, BD Diagnostics) over time and estimated the length of time to preserve clinical vaginal specimens. The ATTS collection system swab results were within 10% range of the control swabs. *G vaginalis* exhibited the greatest increase in sensitivity versus the control at time zero with 81% to 90% total percent positive.²⁸ DNA hybridization was significantly more likely than wet mount to identify *G vaginalis* (45% versus 14%) and *Candida* spp (11% versus 7%). Symptomatic women were also more likely to have positive DNA hybridization findings than asymptomatic women (23% versus 10%).²⁹ DNA hybridization is more sensitive than conventional wet mount and easier to perform.³⁰ Benefits of DNA hybridization over office-based wet mount are that many clinicians do not have the time to perform a wet mount, or may be inexperienced in wet mount interpretation and it improves diagnosis of coinfection, which allows for implementation of appropriate and more cost-effective treatment. Thus, DNA hybridization can be used to improve diagnostic accuracy at the point of care, especially in cases of concomitant *T vaginalis* and BV.³⁰

Cultures for both yeast and *Trichomonas* require incubation, delaying results for 24 to 48 hours. However, culturing may have some value for patients with symptoms of vaginitis when traditional wet-mount findings are negative and DNA hybridization is not available.

MANAGEMENT

Improved diagnostic modalities for vaginitis can promote more efficient management strategies, patient satisfaction, and cost savings. However, due to convenience issues, clinicians do not always include office-based testing.

Over-the-counter antifungal medications for candidiasis are widely used despite the inaccuracy of self-diagnosis for yeast infection.²¹ Oral and topical antimycotic drugs achieve comparable clinical cure rates for women with uncomplicated symptomatic yeast vaginitis.³¹ In a study comparing a single, oral dose of fluconazole to 7 days of vaginal/topical therapy, similar cure rates were achieved for uncomplicated yeast infections.³² Fluconazole, 150 mg as a single dose, is the only oral azole currently approved by

Coding for Vaginitis

82120	Amines (vaginal fluid) qualitative
83986	pH for body fluid, except blood
87480	Infectious agent detection by nucleic acid (DNA or RNA); <i>Candida</i> spp, direct-probe technique
87510	Infectious agent detection by nucleic acid (DNA or RNA); <i>Gardnerella vaginalis</i> , direct-probe technique
87797	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct-probe technique, each organism

the US Food and Drug Administration. As the incidence of recurrent vaginitis is higher in immunocompromised women, patients with repeated infections should be screened for HIV. Such cases may require a 6-month course of antifungal therapy.

Women with complicated yeast infections include those with poorly controlled diabetes, immunosuppression, severe signs/symptoms, and infection with *C glabrata*.

Azole therapy for *C glabrata* has a treatment failure rate of approximately 50%.³³ These cases may require a longer course of topical antimycotic therapy (7 to 14 days) or two doses of oral azole therapy (72 hours apart).³⁴

The standard treatment for trichomoniasis is metronidazole. In randomized trials, a single, 2-g oral dose achieved cure rates of 90% to 95%.³⁴ Reinfection is likely unless the patient's partner is treated as well. Tinidazole is an option for infections resistant to metronidazole. Multiple studies have shown no association between metronidazole use in pregnancy and birth defects.³⁵ However, data do not show that treatment of asymptomatic women with *Trichomonas* during pregnancy lessens the risk of preterm birth.³⁶

As BV has been associated with postoperative PID, endometritis, and cellulitis following gynecologic surgery, preoperative screening and therapy are advised. Although pregnant women with BV may be at increased risk for preterm birth,^{37,38} there are conflicting data as to whether treatment is beneficial for low-risk, asymptomatic women.³⁹

Finally, proper coding is an essential component of office-based evaluation and management of vaginitis. This is especially important to clarify the course of disease and therapy in cases of pregnancy and repeated, complicated, or concomitant infections.

CONCLUSION

Symptomatic vaginitis is a common problem among reproductive-aged women that incurs a substantial cost. Using proper office diagnostic techniques improves

patient satisfaction and ultimately reduces the cost of care. Appropriate office testing can improve the diagnostic accuracy for candidiasis, trichomoniasis, and BV—providing coverage for the majority of vaginitis cases.

REFERENCES

- Kent HL. Epidemiology of vaginitis. *Am J Obstet Gynecol.* 1991;165(4 pt 2):1168-1176.
- Landers DV, Wiesenfeld HC, Heine RP, Krohn MA, Hillier SL. Predictive value of the clinical diagnosis of lower genital tract infection in women. *Am J Obstet Gynecol.* 2004;190(4):1004-1010.
- Drake TE, Maibach HI. Candida and candidiasis. 1. Cultural conditions, epidemiology, and pathogenesis. *Postgrad Med.* 1973;53(2):83-87.
- Allen-Davis JT, Beck A, Parker R, Ellis JL, Polley D. Assessment of vulvovaginal complaints: accuracy of telephone triage and in-office diagnosis. *Obstet Gynecol.* 2002;99(1):18-22.
- Hurley R. Invertebrate vaginal thrush. *Practitioner.* 1975;215(1290):753-757.
- Horowitz B, Edelstein SW, Lippman L. Sexual transmission of Candida. *Obstet Gynecol.* 1987;69(6):883-886.
- Beigi RH, Meyn LA, Moore DM, Krohn MA, Hillier SL. Vaginal yeast colonization in nonpregnant women: a longitudinal study. *Obstet Gynecol.* 2004;104(5 pt 1):926-930.
- Sobel JD. Vaginitis. *N Engl J Med.* 1997;337(26):1896-1903.
- Wendel KA, Erbeling EJ, Gaydos CA, Rompalo AM. Trichomonas vaginalis polymerase chain reaction compared with standard diagnostic and therapeutic protocols for detection and treatment of vaginal trichomoniasis. *Clin Infect Dis.* 2002;35(5):576-580.
- Cates W Jr. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. American Social Health Association Panel. *Sex Transm Dis.* 1999;26(4 suppl):S2-S7.
- Kott H, Adler S. A serological study of Trichomonas sp. parasitic in man. *Trans R Soc Trop Med Hyg.* 1961;55:333-344.
- Robinson SC, Mirchandani G. Observations on vaginal trichomoniasis. IV. Significance of vaginal flora under various conditions. *Am J Obstet Gynecol.* 1965;91:1005-1012.
- Faro S. Sexually Transmitted Diseases in Women. 2003; Lippincott, Williams and Wilkins: Philadelphia, PA.
- Moodley P, Wilkinson D, Connolly C, Moodley J, Sturm AW. Trichomonas vaginalis is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. *Clin Infect Dis.* 2002;34(4):519-522.
- Cotch MF, Pastorek JG, Nugent RP, et al. Trichomonas vaginalis associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. *Sex Transm Dis.* 1997;24(6):353-360.
- Goldenberg RL, Klebanoff MA, Nugent R, Krohn MA, Hillier S, Andrews WW. Bacterial colonization of the vagina during pregnancy in four ethnic groups. Vaginal Infections and Prematurity Study Group. *Am J Obstet Gynecol.* 1996;174(5):1618-1621.
- Gutman RE, Peipert JF, Weitzen S, Blume J. Evaluation of clinical methods for diagnosing Bacterial vaginosis. *Obstet Gynecol.* 2005;105(3):551-556.
- Faro S, Martens M, Maccato M, Hammill H, Pearlman M. Vaginal flora and pelvic inflammatory disease. *Am J Obstet Gynecol.* 1993;169(2 pt 2):470-474.
- Martin HL, Richardson BA, Nyange PM, et al. Vaginal lactobacilli, microbial flora and the risk of human immunodeficiency virus type I and sexually transmitted disease acquisition. *J Infect Dis.* 1999;180(6):1863-1868.
- Sobel JD. Vaginal infections in adult women. *Med Clin North Am.* 1990;74(6):1573-1602.
- Ferris DG, Nyirjesy P, Sobel JD, Soper D, Pavletic A, Litaker MS. Over-the-counter antifungal drug misuse associated with patient-diagnosed vulvovaginal candidiasis. *Obstet Gynecol.* 2002;99(3):419-425.
- Kurth A, Whittington WL, Golden MR, Thomas KK, Holmes KK, Schwebke JR. Performance of a new, rapid assay for detection of Trichomonas vaginalis. *J Clin Microbiol.* 2004;42(7):2940-2943.
- Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med.* 1983;74(1):14-22.
- Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol.* 1991;29(2):297-301.
- Mastrobattista JM, Bishop KD, Newton ER. Wet smear compared with gram stain diagnosis of bacterial vaginosis in asymptomatic pregnant women. *Obstet Gynecol.* 2000;96(4):504-506.
- Bogges KA, Trevett TN, Madianos PN, et al. Use of DNA hybridization to detect vaginal pathogens associated with bacterial vaginosis among asymptomatic pregnant women. *Am J Obstet Gynecol.* 2005;193(3 pt 1):752-756.
- Briselden AN, Hillier SL. Evaluation of Affirm VP Microbial Identification Test for Gardnerella vaginalis and Trichomonas vaginalis. *J Clin Microbiol.* 1994;32(1):148-152.
- Brown HL, Fuller DA, Davis TE, Schwebke JR, Hillier SL. Evaluation of the Affirm Ambient Temperature Transport System for the detection and identification of Trichomonas vaginalis, Gardnerella vaginalis, and Candida species from vaginal fluid specimens. *J Clin Microbiol.* 2001;39(9):3197-3199.
- Brown HL, Fuller DD, Jasper LT, Davis TE, Wright JD. Clinical evaluation of Affirm VPIII in the detection and identification of Trichomonas vaginalis, Gardnerella vaginalis, and Candida species in vaginitis/vaginosis. *Inf Dis Obstet Gynecol.* 2004;12(1):17-21.
- Krieger JN. Urologic aspects of trichomoniasis. *Invest Urol.* 1981;18(8):411-417.
- Watson MC, Grimshaw JM, Bond CM, Mollison J, Ludbrook A. Oral versus intra-vaginal imidazole and triazole anti-fungal treatment of uncomplicated vulvovaginal candidiasis (thrush). *Cochrane Database Syst Rev.* 2001;(4):CD002845.
- Sobel JD, Brooker D, Stein GE, et al. Single oral dose fluconazole compared with conventional clotrimazole topical therapy of Candida vaginitis. Fluconazole Vaginitis Study Group. *Am J Obstet Gynecol.* 1995;172(4 pt 1):1263-1268.
- Sobel JD, Chaim W. Treatment of Torulopsis glabrata vaginitis: a retrospective review of boric acid therapy. *Clin Infect Dis.* 1997;24(4):649-652.
- Sexually transmitted diseases treatment guidelines 2002. Centers for Disease Control and Prevention. *MMWR Recomm Rep.* 2002;51(RR-6):1-78.
- Caro-Paton T, Carvajal A, Martin de Diego I, Martin-Arias LH, Alvarez Requejo A, Rodriguez Pinilla E. Is metronidazole teratogenic? A meta-analysis. *Br J Clin Pharmacol.* 1997;44(2):179-182.
- Klebanoff MA, Carey JC, Hauth JC, et al. Failure of metronidazole to prevent preterm delivery among pregnant women with asymptomatic Trichomonas vaginalis infection. *N Engl J Med.* 2001;345(7):487-493.
- Hauth JC, Goldenberg RL, Andrews WW, DuBard MB, Copper RL. Reduced incidence of preterm delivery with metronidazole and erythromycin in women with bacterial vaginosis. *N Engl J Med.* 1995;333(26):1732-1736.
- McDonald HM, O'Loughlin J, Vigneswaran R, et al. Impact of metronidazole therapy on preterm birth in women with bacterial vaginosis flora (Gardnerella vaginalis): a randomised, placebo controlled trial. *Br J Obstet Gynaecol.* 1997;104(12):1391-1397.
- Carey JC, Klebanoff MA, Hauth JC, et al. Metronidazole to prevent preterm delivery in pregnant women with asymptomatic bacterial vaginosis. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *N Engl J Med.* 2000;342(8):534-540.