

# A Prospective, Randomized, Double-Blind Study of Vaginal Microflora and Epithelium in Women Using a Tampon with an Apertured Film Cover Compared with Those in Women Using a Commercial Tampon with a Cover of Nonwoven Fleece<sup>∇</sup>

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**Healthy women with normal menstrual cycles were randomly assigned to use either a test tampon during cycle 1 and a reference tampon during cycle 2 or a reference tampon during cycle 1 and a test tampon during cycle 2. Tampons were identical except for their cover materials: apertured film for the test tampon and nonwoven fleece for the reference tampon. Product use was doubly blinded. Qualitative and quantitative analyses of vaginal cultures were done pre-, mid-, and postmenstrually for a broad panel of microorganisms, colposcopy was performed, and diary reports were collected; 101 of 105 enrolled subjects completed the study. Midmenstrual findings for a variety of organisms differed from pre- and postmenstrual observations whether subjects were using test or reference tampons. No statistically significant differences were noted in prevalence or colony counts at premenstrual versus mid- and postmenstrual visits for most microorganisms. Prevalences of *Gardnerella* and anaerobic gram-negative rods were significantly different between tampons at the premenstrual visit, when unusually low values were observed for the test and reference tampons, respectively. None of the changes or differences in microflora were considered to be clinically significant. It is noteworthy, however, that declines in the prevalence and abundance of *Lactobacillus* during the menstrual periods were less pronounced during the use of both test and reference tampons than those reported from previous studies. Colposcopy showed no abnormal findings with either tampon and no changes in vaginal or cervical epithelial integrity. Thus, all evidence from both microbiological and colposcopic evaluations indicates that the apertured film cover of the test tampon is as safe as the nonwoven cover of the reference tampon.**

Commercial intravaginal menstrual tampons have been widely used by women since the 1930s. Billions of tampons are sold every year, with an estimated 50% to 70% of women in industrialized countries using them. Underlying public acceptance is the safety of tampons, which has been demonstrated repeatedly in studies involving microbiological analyses, gynecological examination, and subject evaluation (7, 13, 17). Tampons have been shown to not substantially alter the vaginal microflora, and studies of vaginal microflora at various times during the menstrual cycle have demonstrated that tampons have no significant effect on the normal changes that occur in prevalences and total colony counts of either aerobic or anaerobic organisms during menses (10, 14, 15, 16).

The regulatory requirements for the marketing of tampons vary worldwide. One of the most stringent regulatory bodies is the U.S. Food and Drug Administration (FDA), which classifies tampons as medical devices (class II, special controls) and requires preclinical and clinical function and safety studies in

support not only of new tampon products but also of significant changes in the material or design of an already marketed tampon (5). Similar regulations exist in Canada and Australia. In other countries, including those in the European community, tampons are classified as commodities or consumer products, and clinical studies are not required for premarket approval. In Europe, the *European General Product Safety Directive* (4) and the *European Code of Practice for Tampons* (defined by the European Disposables and Nonwovens Association, an international trade association of absorbent hygiene product industries that, among other things, establishes standards for hygiene products) (3) are followed. Each manufacturer decides whether it is necessary to conduct a clinical trial to confirm the safety of significant changes in tampon materials or design (4).

Marketed tampons are currently made of rayon, cotton, or cotton-rayon fiber blends, and most tampons have an overwrap of lightweight nonwoven fleece. The degree of absorbency for each tampon, defined as grams of fluid absorbed using standardized laboratory test methods (3, 9), allows different tampon brands to be compared by both regulatory authorities and consumers. Although many tampon brands, levels of absorbency, and styles are now available, some manufacturers continue to further develop tampons in order to enhance comfort and ease of use.

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The o.b. tampon was first introduced in Germany in 1950, and a nonwoven fleece cover has been used in o.b. tampons since 1982. The normal-absorbency version (European *Code of Practice for Tampons* three-droplet category) of this tampon with an eight-groove design (the tampon that served as the reference tampon for this clinical trial) has been widely (billionfold) used since its introduction in 1992. Recently, a new tampon variant with an apertured film cover to permit fluid to flow into the core of the tampon while affording more comfortable insertion and removal, especially during light menstrual flow, was designed (Johnson & Johnson Consumer and Personal Products) (unpublished data). The current prospective, randomized clinical trial was undertaken to confirm the safety of this new tampon design with respect to vaginal microflora and vaginal and cervical epithelium. The data were also expected to improve our understanding of changes in vaginal microflora throughout the menstrual cycle.

#### MATERIALS AND METHODS

**Study design.** This double-blind, randomized, two-way crossover trial was conducted at three centers in Germany according to FDA regulations and the International Conference on Harmonisation Guidelines for Good Clinical Practice (4a). All subjects were fully informed and provided written consent for participation prior to enrollment. The protocol was reviewed and approved by the Ethik-Kommission bei der Landesärztekammer Hessen, Institutional Ethics Committee, Frankfurt, Germany.

**Subjects.** Study participants were healthy women, 18 to 45 years of age, with normal menstrual cycles (21 to 35 days) who regularly used o.b. normal-absorbency tampons (or other brands of similar absorbency [three droplets according to the European *Code of Practice for Tampons*]) (3) for at least 4 days of their menses. At a screening visit, subjects underwent a gynecological examination, including vaginal and cervical colposcopy, and the vaginal pH was determined. Vaginal samples were collected to perform Gram staining (12) and microbiological cultures, and a Papanicolaou (Pap) smear was done unless results from a test performed within the prior 6 months were available. A physical examination was performed, and vital signs (blood pressure, heart rate, and temperature), menstrual and medical histories, and concomitant medications were recorded.

Subjects were eligible if they had no vaginal or cervical irritation and/or vaginal dryness upon colposcopic examination at the screening visit. Additional inclusion criteria were a documented normal pelvic examination, including Pap smear (at or within 6 months of screening), vaginal pH of  $\leq 4.5$  ( $< 5.0$  if the subject was using a hormonal contraceptive), and a Gram stain score of  $< 7$  (12). Exclusion criteria included the following: vaginal, perineal, or urinary tract infection or inflammation; sexually transmitted disease or vaginitis currently or within the previous 2 months; treatment for or suspicion of ever having had toxic shock syndrome; history of vulvar, vaginal, or cervical dysplasia, neoplasia, and/or cancer, condyloma, or human papillomavirus within the previous 2 years; positive test for human immunodeficiency virus; regular use of systemic corticosteroids or current treatment for infection; regular urinary incontinence; history of genital herpes, pelvic radiation therapy, or diabetes mellitus; and pregnancy or lactation.

**Study tampons and procedures.** Eligible subjects were assigned to one of two "treatment" sequences according to a computer-generated schedule provided by an independent research organization: (i) test tampon during cycle 1 and reference tampon during cycle 2 (test-reference group) and (ii) reference tampon during cycle 1 and test tampon during cycle 2 (reference-test group). Neither investigators nor subjects were aware of which sequence was assigned. The reference and test tampons were identical except for their covers, and both were made with a blend of two rayon fibers. The reference tampon, commercial o.b. available in Europe, had a nonwoven fleece cover. The test tampon had a cover made of apertured film. Subjects were instructed to wear tampons as they would customarily do but not to wear an individual tampon for longer than 8 h. They were also advised to use the assigned study tampon continuously and exclusively for at least 4 days. For backup menstrual protection, sanitary napkins (Stayfree Ultrathin Plus; Personal Products Company, Skillman, NJ) were provided. Subjects were instructed to refrain from vaginal douching; using feminine deodorant sprays or any topical over-the-counter products in the perineal/vaginal area for the duration of the study; and using contraceptive foams, gels, creams, sponges,

or devices such as diaphragms and condoms during menstruation and for 2 days prior to each examination.

**Study assessments.** During the study, assessments were performed at three visits during each cycle: a premenstrual visit during days  $-10$  to  $-1$ , considering day 1 to be the first day of menstruation; a midmenstrual visit during days 2 to 4, after the use of two or more tampons for a total of at least 12 h; and a postmenstrual visit during days 7 to 12, at least 48 h after use of the last tampon. At the randomization visit, participants received the assigned tampons for cycle 1 and were given diary cards to record tampon use, concomitant medications, and the occurrence of medical problems. The assigned tampons for cycle 2 and diary cards were distributed at the cycle 2 premenstrual visit. To maintain blinding, study tampons for both cycles were provided in identical, unmarked cellophane overwraps and were packaged in identical boxes.

At each pre-, mid-, and postmenstrual visit, vital signs, adverse events, and a review of concomitant medication were recorded. A speculum examination was performed, and vaginal swabs were collected for qualitative and quantitative microbiological evaluation. At each postmenstrual visit, diary cards were collected, and a colposcopic examination was performed to evaluate cervical mucosa and vaginal epithelium for irritation, dryness, edema, erythema, micro-ulceration, erosion, inflammation, infection, and tampon fiber retention. A final physical examination was conducted at the cycle 2 postmenstrual visit. Throughout the study, subjects were instructed to contact the investigator if they experienced any adverse reactions, especially pelvic pain, perineal or vaginal itching, burning or abnormal discharge, or signs or symptoms of toxic shock syndrome.

Subjects were asked to make every effort to use the study tampons correctly for two consecutive menstrual periods. If complete data were not available for any one cycle and/or the subject was unable to complete a cycle for personal or medical reasons, the investigator could allow this cycle to be repeated at the end of the study (cycle 3) using the appropriate tampon according to the randomization schedule. If any data were missing for two cycles or if the subject was not compliant with study tampon use for two cycles, the subject was withdrawn from the study.

**Microbiology analyses.** At screening, to determine the presence of vaginosis, vaginal swabs were obtained for Gram staining and quantitative culture. The standardized method for the interpretation of Gram-stained vaginal smears developed previously by Nugent et al. (12) was used; a score of 7 to 10 confirmed the diagnosis of bacterial vaginosis.

At the pre-, mid-, and postmenstrual visits, microorganisms were identified (*Lactobacillus* species, *Staphylococcus aureus*, *Escherichia coli*, group B *Streptococcus*, *Gardnerella vaginalis*, anaerobic gram-negative rods, *Enterococcus* species, *Candida albicans*, and *Candida* species), mean colony counts (CFU/ml) per vaginal secretion were obtained, and the prevalence of each microorganism was calculated. Vaginal smears were obtained using two sterile Dacron-tipped swabs rolled against the lateral wall of the vagina until saturation. The capacity of these swabs was previously determined by Hillier et al. (6) to be 0.1 ml by measuring the weight of vaginal fluid in 10 volunteers. The swabs were placed in a BBL Port-A-Cul transport device (Becton Dickinson, Heidelberg, Germany) and were shipped to the Laboratory Group Heidelberg Department of Microbiology in Heidelberg, Germany, on the same day. The two swabs (containing 0.2 ml of vaginal secretions) were inoculated into 1.8 ml Hanks' medium (1:10 dilution). Serial dilutions of 1:10 from  $10^{-1}$  to  $10^{-4}$  were prepared in sterile saline solution. One hundred microliters of each solution was plated onto a series of different agar media: Columbia blood agar (Becton Dickinson) with 5% sheep blood for growth of *S. aureus*, *Enterococcus* species, and group B *Streptococcus*; MacConkey agar (Oxoid, Basingstoke, Hampshire, England) for *E. coli*; Rogosa agar (Becton Dickinson) for *Lactobacillus* species; Schädler K/V agar (Becton Dickinson) for anaerobic gram-negative rods; Chromagar (Mast Diagnostics, Bootle, Merseyside, United Kingdom) for *Candida* species; and *Gardnerella vaginalis* agar (Heipha, Heidelberg, Germany) for *G. vaginalis*. Isolates were identified using criteria established previously by Murray et al. (11). Colony counts were determined by an enumeration of the mean number of colonies of each species multiplied by the respective dilutions.

**Statistical analyses.** Because the study was a balanced, crossover trial, statistical analyses focused on the per-protocol population, defined as subjects who completed the study. The analysis of adverse events was performed on the intent-to-treat (ITT) population, which comprised all subjects who were randomized. Statistical methods for categorical data were used to analyze the results of the microbiological tests (8, 21, 22). Prevalence was defined as the percentage of subjects having a vaginal microbe at a single visit. Incidences were assessed by comparing the number and percentage of subjects with the organism present at the mid- or postmenstrual visit but not present at the premenstrual visit. The statistical significance of changes in numbers of subjects with microorganisms from the premenstrual visit to the mid- and postmenstrual visits was analyzed by

TABLE 1. Demographic characteristics (ITT population)

Characteristic	Value		
	Test-reference group (n = 51)	Reference-test group (n = 54)	Total
Age (yr) (mean ± SD)	31.2 ± 7.49	32.0 ± 7.74	31.6 ± 7.60
Range	18–45	18–45	18–45
Menstrual flow [no. of women (%)]			
Heavy	6 (11.8)	2 (3.7)	8 (7.6)
Moderate	45 (88.2)	52 (96.3)	97 (92.4)
Light	0 (0)	0 (0)	0 (0)
Absorbency of tampons <sup>a</sup> normally used [no. of women (%)] <sup>b</sup>			
Normal	51 (100)	54 (100)	105 (100)
Mini	8 (15.7)	10 (18.5)	18 (17.1)
Super	7 (13.7)	5 (9.3)	12 (11.4)
Super plus	0 (0)	0 (0)	0 (0)
Days per cycle (mean ± SD)	27.8 ± 1.26	28.0 ± 1.10	27.9 ± 1.18
Range	23–29	24–31	23–31
Days of flow (mean ± SD)	5.0 ± 0.75	5.1 ± 0.75	5.1 ± 0.75
Range	4–7	4–8	4–8

<sup>a</sup> Tampon absorbency denominations correspond to tampon absorbency (“droplet”) categories of the European Disposables and Nonwovens Association’s tampon code of practice (3) as follows: “mini,” 2 droplets (6- to 9-g absorbency); “normal,” 3 droplets (9- to 12-g absorbency); “super,” 4 droplets (12- to 15-g absorbency); “super plus,” 6 droplets (18- to 21-g absorbency). The first three of these correspond roughly to “regular,” “super,” and “super plus” absorbencies, respectively, according to FDA labeling requirements in the United States; absorbencies higher than 18 g are not covered by U.S. regulations (9).

<sup>b</sup> Total percentage figures could exceed 100% because respondents could report using more than one absorbency of tampon.

the McNemar test ( $\alpha = 0.05$ ; two-sided test). *Lactobacillus* species, *S. aureus*, *E. coli*, group B *Streptococcus*, *G. vaginalis*, anaerobic gram-negative rods, *Enterococcus* species, *C. albicans*, and *Candida* species were evaluated using both continuous and categorical models (8, 21, 22). The continuous variable analysis assessed the mean change of each microorganism count from the premenstrual visit to the midmenstrual visit performed on a logarithmic scale. An identical comparison was made using the change from the premenstrual visit to the postmenstrual visit. A preliminary analysis of the difference in carryover effects was assessed by an analysis of variance model with terms for sequence, subject within sequence, period, and treatment group. Because there was no carryover effect from cycle 1 to cycle 2, the results obtained for each tampon in cycle 1 were combined with those obtained for the same tampon in cycle 2, and data from the two tampon products were compared. The colposcopic results were analyzed by the categorical method, and since the responses for these variables were ordinal, cumulative logits were used to code them. Adverse events were analyzed using descriptive statistics; statistical comparisons using the Fisher exact or chi-square test were performed if indicated by the occurrence of an appropriate number of events.

Although a considerable number of statistical tests were to be performed, the analysis and interpretation of the results were to be mostly descriptive. In addition, no single microorganism, or other variable formed using a combination of single microorganisms, was considered to be the primary variable of clinical importance upon which to base sample size estimates for statistical purposes. Therefore, sample size was not based on statistical calculation but rather on previous experience with health care consultants and regulatory authorities (510[k] submissions to the FDA for changes in tampon composition), which indicated that a sample size of at least 40 subjects with evaluable data from both cycles of tampon use would yield credible results. The total numbers of subjects to be screened and randomized were set at 120 and 90, respectively, based on experience with subject discontinuation in previous tampon studies and the expected dropout rate given the study’s precisely scheduled assessments within the course of the menstrual cycle.

RESULTS

**Demographic characteristics and subject disposition.** A total of 105 women were enrolled in the study and randomized (ITT population) between 4 September and 2 October 2000; the last subject completed the study on 13 November 2000. Fifty-one women were randomly placed into the test-reference group, and 54 women were randomly placed into the refer-

ence-test group. Nearly all enrollees in both groups were Caucasian, and age distributions were similar across treatment sequences (Table 1). There was little variation in menstrual flow, absorbency of tampons normally used, cycle length, and duration of flow across treatment sequences (Table 1).

In all, 101 of the 105 (96.2%) enrolled subjects completed the study (50 subjects in the test-reference group and 51 subjects in the reference-test group). Discontinuations were due to adverse events unrelated to the product ( $n = 2$  for reference-test group) and noncompliance ( $n = 1$  for each treatment group). Major protocol violations occurred in 7 subjects in the test-reference group and in 14 subjects in the reference-test group. Major protocol violations were questionable diary entries ( $n = 6$ ); samples for microbiological culture being too old, showing no growth, or being lost ( $n = 5$ ); visits not attended or out of the allowable time window ( $n = 5$ ); tampon noncompliance ( $n = 3$ ); and antibiotic comedication ( $n = 3$ ). The per-protocol population included 43 subjects in the test-reference group and 40 subjects in the reference-test group. In both groups, 65% of patients reported using contraception (per-protocol population).

**Microbiology results.** Table 2 summarizes the prevalence and average colony counts for each tampon and indicates the statistical significance of comparisons within and between tampons.

**Sequential changes in vaginal microflora during menstrual cycles.** Midmenstrual findings for the vaginal microflora differed from those of pre- and postmenstrual observations for a variety of organisms whether subjects were using test or reference tampons. For midmenstrual versus premenstrual contrasts, prevalences and colony counts were generally lower for the *Lactobacillus* species and higher for *S. aureus*, *E. coli*, group B *Streptococcus*, and anaerobic gram-negative rods; several but not all contrasts reached statistical significance for within-tampon comparisons. Colony counts for *G. vaginalis* were higher



TABLE 2. Prevalences and descriptive statistics of colony counts (per-protocol population)

Microorganism	Test tampon (n = 83)				Reference tampon (n = 83)							
	Premenstrual <sup>a</sup>		Midmenstrual		Postmenstrual		Premenstrual <sup>a</sup>		Midmenstrual		Postmenstrual	
	Prevalence <sup>b</sup> [no. (%)]	Colony count <sup>c</sup> (mean log <sub>10</sub> CFU/ml ± SD)	Prevalence <sup>b</sup> [no. (%)]	Colony count <sup>c</sup> (mean log <sub>10</sub> CFU/ml ± SD)	Prevalence <sup>b</sup> [no. (%)]	Colony count <sup>c</sup> (mean log <sub>10</sub> CFU/ml ± SD)	Prevalence <sup>b</sup> [no. (%)]	Colony count <sup>c</sup> (mean log <sub>10</sub> CFU/ml ± SD)	Prevalence <sup>b</sup> [no. (%)]	Colony count <sup>c</sup> (mean log <sub>10</sub> CFU/ml ± SD)	Prevalence <sup>b</sup> [no. (%)]	Colony count <sup>c</sup> (mean log <sub>10</sub> CFU/ml ± SD)
<i>Lactobacillus</i> species	82 (98.8)	7.4 ± 1.38	74 (89.2) <sup>d</sup>	7.1 ± 1.04	79 (95.2)	7.3 ± 1.16	81 (97.6)	7.7 ± 1.05	74 (89.2) <sup>d</sup>	7.1 ± 0.84 <sup>d</sup>	82 (98.8)	7.2 ± 1.15 <sup>d</sup>
<i>S. aureus</i>	9 (10.8)	3.9 ± 0.82	22 (26.5) <sup>d</sup>	5.3 ± 1.74 <sup>d</sup>	11 (13.3)	3.6 ± 1.10	8 (9.6)	4.0 ± 1.33	23 (27.7) <sup>d</sup>	5.4 ± 1.54 <sup>d</sup>	13 (15.7)	3.6 ± 1.09
<i>E. coli</i>	11 (13.3)	3.3 ± 1.64	43 (51.8) <sup>d</sup>	5.6 ± 1.71 <sup>d</sup>	24 (28.9) <sup>d</sup>	3.4 ± 1.77	16 (19.3)	4.0 ± 1.63	42 (50.6) <sup>d</sup>	5.5 ± 1.70 <sup>d</sup>	19 (22.9)	3.6 ± 1.30
Group B <i>Streptococcus</i>	2 (2.4)	5.6 ± 0.76	17 (20.5) <sup>d</sup>	6.3 ± 1.71	10 (12.0) <sup>d</sup>	4.8 ± 2.11	4 (4.8)	4.6 ± 2.77	21 (25.3) <sup>d</sup>	6.6 ± 1.26	11 (13.3)	4.8 ± 1.63
<i>G. vaginalis</i>	3 (3.6) <sup>e</sup>	6.3 ± 1.60	13 (15.7) <sup>d</sup>	6.6 ± 1.17	14 (16.9) <sup>d</sup>	6.9 ± 1.75	16 (19.3) <sup>e</sup>	6.6 ± 1.94	14 (16.9)	7.0 ± 1.04	19 (22.9)	7.1 ± 1.73
Anaerobic gram-negative rods	20 (24.1) <sup>f</sup>	3.4 ± 1.10	32 (38.6) <sup>d</sup>	4.3 ± 1.26 <sup>d</sup>	16 (19.3)	3.8 ± 1.46	6 (7.2) <sup>f</sup>	3.3 ± 0.89	35 (42.2) <sup>d</sup>	4.4 ± 1.40 <sup>d</sup>	22 (26.5) <sup>d</sup>	3.8 ± 1.87
<i>Enterococcus</i> species	12 (14.5)	4.1 ± 1.33	14 (16.9)	4.7 ± 1.42	13 (15.7)	3.8 ± 1.13	12 (14.5)	4.2 ± 1.52	14 (16.9)	5.0 ± 1.23	12 (14.5)	3.5 ± 1.16
<i>C. albicans</i>	9 (10.8)	3.9 ± 1.27	14 (16.9)	4.1 ± 1.40	7 (8.4)	3.2 ± 1.28	10 (12.1)	3.8 ± 0.87	15 (18.1)	3.9 ± 1.53	8 (9.6)	3.1 ± 1.07
<i>Candida</i> species	3 (3.6)	2.8 ± 1.00	2 (2.4)	3.3 ± 0.48	2 (2.4)	1.8 ± 0.34	2 (2.4)	2.5 ± 0.29	3 (3.6)	3.3 ± 1.15	3 (3.6)	3.2 ± 0.56

<sup>a</sup> The number of subjects at premenstrual visits is too small for statistical testing for *S. aureus* (reference tampon), group B *Streptococcus* (test and reference tampon), *G. vaginalis* (test tampon), anaerobic gram-negative rods (reference tampon), and *Candida* species (test and reference tampon).

<sup>b</sup> Prevalences are given as the absolute number of subjects and as a percentage of the per-protocol population.

<sup>c</sup> Mean colony counts expressed as log<sub>10</sub> CFU/ml of vaginal secretion.

<sup>d</sup> *P* < 0.05 for difference within test or reference tampons compared to each corresponding premenstrual time point.

<sup>e</sup> *P* < 0.05 for difference between test and reference tampons at the premenstrual time point.

for both tampons at midmenstrual visits than at premenstrual visits, but the prevalence of this organism increased only for the test tampon. The menstrual cycle appeared to have little effect on prevalence rates or colony counts for *Enterococcus* species, *C. albicans*, and other *Candida* species. For both test and reference tampons, colony counts remained within the ranges reported by investigators cited above throughout the course of the study for all microorganisms assessed.

**Differences between tampons with regard to vaginal microflora.** No statistically significant differences between test and reference tampons were noted with regard to prevalence or colony counts at the premenstrual versus the mid- and postmenstrual visits for most of the microorganisms evaluated, including *Lactobacillus* species, *S. aureus*, *E. coli*, group B *Streptococcus*, *Enterococcus* species, *C. albicans*, and other *Candida* species. The prevalence of *E. coli* increased significantly between the premenstrual and the midmenstrual visits for both tampons, but prevalence remained significantly elevated at the postmenstrual evaluation only for the test tampon. Statistically significant differences between the two tampons were noted in the prevalence of *G. vaginalis* and anaerobic gram-negative rods at the premenstrual visit. The premenstrual prevalence of *G. vaginalis* was unusually low for the test tampon, while that of gram-negative rods was unusually low for the reference tampon. In both cases, increases in prevalence from the premenstrual visit to the mid- and postmenstrual visits were statistically significant.

**Incidence.** New occurrences at the midmenstrual visit not present at the premenstrual visit were noted for most organisms. The highest such incidences were seen for *S. aureus*, *E. coli*, group B *Streptococcus*, and anaerobic gram-negative rods. The number of subjects with a particular microorganism at the midmenstrual visit or the postmenstrual visit but not at the premenstrual visit did not differ significantly between tampons except in the case of anaerobic gram-negative rods. The number of those with anaerobic gram-negative rods at the post- but not the premenstrual visit was significantly higher for the reference than for the test tampon.

**Colposcopy results.** Colposcopic examinations of both the ITT and per-protocol populations showed no abnormal findings after use of either test or reference tampons (data not shown).

**Summary of tampon use.** Subject diaries revealed no clinically relevant differences in tampon use either between subjects wearing each tampon or between those allocated to the two treatment sequences (Table 3). For both cycles and both tampons, the average time that a single tampon was used was approximately 5 h, and the maximum total time of tampon use was approximately 100 h. Of the 83 subjects in the per-protocol population, 18 deviated from the protocol by using a tampon for more than 8 h. These subjects were not excluded from the final evaluations because such variations were not considered to be major protocol deviations and because reporting complete results for such subjects was considered to be especially important, given the common concern about the safety of a tampon remaining in use for longer than 8 h. In general, women used between 11 and 30 tampons per cycle, with a median of 20 tampons.

**Adverse events.** Of the 105 subjects included in the ITT population, 8 of 51 (15.7%) reported 10 adverse events in the

TABLE 3. Summary of tampon use per subject (per-protocol population)

Parameter	Cycle	Value for treatment sequence	
		Test-reference (n = 43)	Reference-test (n = 40)
Average time of single tampon use in h:min (mean ± SD)	1	4:53 ± 1:03	5:01 ± 0:57
	2	4:57 ± 0:59	4:57 ± 0:54
Maximum total amt of time of tampon use per cycle in h:min (mean ± SD)	1	96:01 ± 21:10	98:13 ± 24:22
	2	92:16 ± 19:24	97:09 ± 22:26
No. of tampons used/cycle (mean ± SD)	1	18.70 ± 4.67	18.90 ± 5.09
	2	18.10 ± 4.12	19.30 ± 4.98

test-reference group and 14 of 54 (25.9%) reported 17 adverse events in the reference-test group. No adverse event was judged by the investigators to be related to a study tampon. The majority of adverse events were of mild or moderate intensity, with only one reported to be of severe intensity. No serious adverse events were reported. The most common adverse event was headache observed in four subjects (7.8%) in the test-reference group and in six subjects (11.1%) in the reference-test group. All other adverse events (including migraine, influenza-like symptoms, abdominal pain, spotting between menses, rhinitis, cystitis, and infection) were reported in only one or two individuals per group (1.8% to 3.9%). Two subjects were withdrawn from the study due to the use of proscribed antibiotic treatment for a common cold and cystitis; neither condition was considered to be related to the experimental product. Only minor changes of no clinical relevance in blood pressure, heart rate, and oral temperature were noted throughout the study in both the ITT and the per-protocol populations. No between-tampon differences in vital signs were apparent.

DISCUSSION

This large, randomized, double-blind, crossover study is, to our knowledge, the most rigorous clinical safety study of a new tampon to date and is among the most intensive studies of vaginal microflora changes in relation to menses and tampon use ever done. Assessments included colposcopy, diary reports, and microbiological analyses of specimens collected at three time points (pre-, mid-, and postmenstrual) for a broad panel of potentially clinically significant vaginal microorganisms (seven species or types of bacteria as well as *Candida*). No formal evaluation of the effectiveness of blinding was conducted, but it is highly unlikely that unblinding biased the results for several reasons: (i) randomization was performed by individuals in locations separate from the clinics where subjects were examined, (ii) products looked similar and were packaged identically, (iii) subjects did not have both products at the same time, and (iv) personnel at the microbiology laboratory had no access to the randomization code.

Qualitative and quantitative changes in specific vaginal microflora from pre- to mid- to postmenstrual visits in this study generally were similar between cycles of use of the two differ-

ent tampons and similar to those reported from previous studies of tampon and other catamenial product use (1, 2, 14–16, 19, 20, 23). Thus, there were generally numerical if not statistically significant increases in the prevalence and/or colony counts of anaerobic gram-negative rods, as well as *G. vaginalis*, group B *Streptococcus*, *E. coli*, and *S. aureus*, from the pre- to midmenstrual visits, and these values returned or started to return to the premenstrual values by the postmenstrual visit. These observations are complicated by the inexplicably lower prevalence of *G. vaginalis* at the premenstrual visit before the period of test tampon use and of anaerobic gram-negative rods before the period of reference tampon use. Changes in *Enterococcus* species and *C. albicans* throughout the menstrual cycles were generally qualitatively similar but less pronounced and were virtually identical during the cycles of use of the two different tampons. For all microorganisms assessed, neither the changes during the menstrual cycles nor the differences between cycles of the use of the two tampons were of any clinical significance.

The results for *Lactobacillus* species in the current study also showed no differences between the cycles of use of the two different tampons but are worthy of special note because (i) lactobacilli are the predominant microbes in the healthy vagina and are considered to be responsible for preventing the overgrowth of potentially pathogenic microbes and (ii) the results here differed from those reported in previous studies (2, 16). Eschenbach et al. (2) found that the proportion of women with semiquantitatively high levels of *Lactobacillus* increased from 70% on days 1 to 5 of the cycle to 92% on days 7 to 12. Onderdonk et al. (16) observed that in vaginal swabs from women using tampons during their menstrual periods, *Lactobacillus* prevalence and colony counts increased from day 2 to day 4 to day 21: from 78% to 84% to 91% and from 6.21 to 7.08 to 7.91 log<sub>10</sub> CFU/g, respectively. In the present study, only approximately 10% of the women lost *Lactobacillus* during menses (while using either the test or reference tampons), and among women who retained *Lactobacillus*, there was an average reduction in colony counts of only about a half log. Although the reductions in prevalence were statistically significant during both periods of tampon use and the reduction in colony counts was statistically significant during the period of reference tampon use, these changes were not clinically significant and were much less than those reported in previous studies (2, 16). The differences between our findings and those of previous studies may reflect differences in study populations (e.g., between women in the United States and women in Germany), laboratory methods, or the tampons used in the study. Further studies would be required to evaluate these possibilities.

In the present study, visual pelvic exams found no signs of vaginal or cervical irritation or ulcerations either during or after use of either the test or the reference tampon. Colposcopy after the use of either tampon showed no evidence of microulcerations.

Finally, the analysis of diary records demonstrated that both test and reference tampons were well tolerated. Most reported adverse events were of mild or moderate intensity and were not related to the study tampon. No serious adverse events were reported. The average tampon wear times were similar in both

treatment sequences and were comparable to that reported in another recent study of a new tampon (18).

In conclusion, the findings of this rigorously designed study conducted using a large sample of subjects demonstrate that the new tampon with an apertured film cover has no adverse effects on the vaginal microflora or on the vaginal and cervical epithelium and is as safe and well tolerated as the current commercially marketed tampon with a nonwoven fleece cover, a tampon with a long, extensive history of safe use.

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