

Lactobacillus acidophilus, Strain *NAS* (H₂O₂ Positive) in Reduction of Recurrent Candidal
Vulvovaginitis

Julius Metts, MD¹, Thomas R. Famula, PhD², Roger A. Clemens, DrPH³

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Address correspondence and reprint requests to: Julius Metts, MD, University of California,
Cowell Student Health Center, Davis, CA 95616

Telephone: (530) 752-2300

Fax: (530) 752-2306

E-Mail: jfmetts@student-hlth.ucdavis.edu

¹ University of California, Cowell Student Health Center, Davis, CA 95616

² University of California, Department of Animal Science, Davis, CA 95616

³ California State Polytechnic University, Department of Food Science and Human Nutrition, Pomona, CA 91768

ABSTRACT

An estimated 10 million women each year present bacterial vaginosis (BV). More than 15 million women present yeast vaginitis annually. Several strains of *Lactobacillus acidophilus* are normal flora in many body cavities, including the vaginal tract. These strains produce hydrogen peroxide and lactic acid which are natural antimicrobial products of these strains, and may inhibit the growth of potential pathogens including *Candida albicans* and bacterial organisms, which cause urogenital tract infections. This study examines the safety and potential efficacy of *L. acidophilus* NAS strain vaginal suppositories alone or in combination with oral probiotic strains (*L. acidophilus*, NAS strain; *Bifidobacterium bifidum*, Malyoth strain, and *L. bulgaricus*, LB-51 strain) to reduce the incidence of Candidal vulvovaginitis in women with recurrent candidal vulvovaginitis (4 or more infections in the past 12 months) relative to placebo controls. A triple-blind, randomized, placebo-controlled trial enrolled 34 female graduate and undergraduate students (19-40 years old) for six months during which they were examined for candidal infections while following one of three study regimens. These students, with a history of four or more episodes of candidal vulvovaginitis in the past twelve months, were randomized into one of two treatment groups or a control group. The two treatment groups were: 1) *L. acidophilus* NAS strain (H_2O_2 positive; 5×10^9 cfu) vaginal suppositories with placebo (Solka-Floc™) oral capsules, and 2) *L. acidophilus* NAS strain (H_2O_2 positive; 5×10^9 cfu) vaginal suppositories with probiotic oral capsules (*L. acidophilus*, NAS strain [5×10^9 cfu]; *Bifidobacterium bifidum*, Malyoth strain [20×10^9 cfu], and *Lactobacillus bulgaricus*, LB-51 strain [5×10^9 cfu]). The control group received placebo (Solka-Floc™) vaginally and orally. Oral capsules were self administered daily and vaginal suppositories were self administered three times weekly. Twenty-seven students participated in the trial for an average duration of 3.3 months. The rate of confirmed infections between the two probiotic treatment groups and the placebo control differed

significantly ($P < 0.01$). Documented infections within the first three months of follow up did not differ among all study groups, whereas the average number of infections within the next 3-month period was significantly reduced ($P = 0.032$) between the combined treatment groups and the control. These findings suggest that these probiotic strains provided as vaginal inserts or as oral capsules are safe and may significantly reduce the incidence of candidal vulvovaginitis in women with recurrent infections after 3 months of use.

INTRODUCTION

An estimated 10 million women each year present bacterial vaginosis (BV). More than 15 million women present yeast vaginitis annually. Over 11.4 million women in the United States were treated for urinary tract infections in 1997, at an estimated annual cost of > \$2 billion. Bacterial vaginitis is a serious health risk and is associated with pelvic inflammatory disease,¹ cervicitis,² as well as preterm labor and birth.³ Lactobacilli are the predominant flora in the vagina.⁴ Many lactobacilli produce H₂O₂ and lactic acid, and other natural antimicrobial products, which maintain vaginal acidity (normal pH is 3.8-4.2). These peroxide-producing strains with their antimicrobial agents may suppress bacterial growth of potential pathogens including *Candida albicans* and genital microorganisms, which cause urogenital tract infections.⁵

Some women have recurrent vulvovaginal Candidiasis (RVVC) infections despite the lack of risk factors such as the use of antibiotics, the application of corticosteroids, or the presence of diabetes. Systemic prophylaxis has been reported when the infection in RVVC is caused by *C. albicans*.⁶ For example, an initial 14-day course of oral azole therapy to induce clinical remission followed by a six-month maintenance regimen has been suggested. That regimen included ketoconazole (Nizoral®), 100 mg daily; itraconazole (Sporanox®), 100 mg daily, and fluconazole (Diflucan®), 100 to 200 mg weekly. These therapies have a high medical cost and may present potential adverse side effects, such as jaundice,⁷ hepatotoxic,⁸ nausea, headaches, and diarrhea.^{9,10}

Many hydrogen peroxide positive lactobacilli strains have been isolated from normal healthy, vaginal tracts.⁴ Some of these strains have demonstrated a protective effect against candidal

infection as well as against BV by adhering to the vaginal mucosa and by producing antimicrobials.¹¹

Numerous placebo-controlled studies suggest that preparations of probiotic strains have been successfully used to treat antibiotic associated diarrhea.¹² These include strains of *Lactobacillus casei* GG, *Bifidobacterium longum*, and a combination of strains of *B. longum*, *L. acidophilus* and a strain of *Saccharomyces boulardii*. Other strains have been used to prevent acute rotaviral infantile diarrhea, such as *Bifidobacterium bifidum* and *Streptococcus thermophilus*,¹³ to treat acute recurrent *Clostridium difficile* disease,¹⁴ and to treat various other diarrheal illnesses.^{15,16}

Hilton *et al.* demonstrated that daily consumption of yogurt containing a strain of H₂O₂ positive *Lactobacillus acidophilus* (>10⁸ cfu/mL) significantly reduced the incidence of candidal infections in women with recurrent candidal vulvovaginitis.¹⁷ It is important to note that the U.S. regulatory statutes for yogurt only require the use of *L. bulgaricus* and *S. thermophilus*, without any reference to specific strains. Perhaps more important, when *L. acidophilus* is added to yogurt, there are not any standards to validate that the strain is a H₂O₂ producing organism. Many commercial yogurts contain *L. acidophilus* and *L. casei*, which do not produce hydrogen peroxide.¹⁸

A preliminary study by Hilton *et al.* showed that *Lactobacillus GG* vaginal suppositories self-administered twice a day for seven days reduced the symptoms associated with candidal vaginitis in women with recurrent vulvovaginitis.¹⁹ Prior to this time few clinically controlled studies reported potential efficacy of selected hydrogen peroxide producing strains of lactobacilli vaginal suppositories or oral capsules as therapeutic or prophylactic vehicles directed to candidal

vulvovaginitis in women with recurrent infections. If scientifically validated through additional studies, the application of probiotic preparations may prove to be an inexpensive, efficacious and easy approach for women with recurrent candidal infections or for women who have risk factors for RVVC, including frequent antibiotic therapy, intermittent corticosteroid therapy, diabetes, and pregnancy. Vaginal suppositories may be a more reliable, direct form of establishing a lactobacillus population in the urogenital region than the oral route. Ingested lactobacilli must survive the strong acidic environment of the stomach and then participate in at least a transient colonization of the colon and rectum before translocation to the vaginal area.

This study examines the safety and potential efficacy of *L. acidophilus* NAS strain vaginal suppositories alone or in combination with oral probiotic strains (*L. acidophilus*, NAS strain; *Bifidobacterium bifidum*, Malyoth strain, and *L. bulgaricus*, LB-51 strain) to reduce the incidence of Candidal vulvovaginitis in women with recurrent candidal vulvovaginitis (4 or more infections in the past 12 months) relative to placebo controls.

SUBJECTS

Thirty-four undergraduate and graduate female students (age range: 19-40 years) with recent vaginitis were recruited from a university student health center. Eligible women had a total of four or more candidal vulvovaginal infections in the past twelve months with at least two documented by a medical provider. The study was approved by the institutional review board. All participants provided a signed informed consent which was retained in their respective study file. Participant exclusion criteria included confirmed HIV infection, immunocompromised conditions, pregnancy, and multiple vaginal pathogens. Women receiving

antibiotic therapy were excluded until one month after they had completed therapy. A questionnaire was administered to and completed by each woman at the beginning of the study including information on gynecologic, dietary, medical and sexual history. At admission to the study, each woman was given a pelvic exam. Two cervical cultures were obtained and directly inoculated onto Thayer Martin media or were tested for chlamydia EIA. Two other swabs were taken from the posterior fornix and vaginal walls, inoculated onto blood agar, and examined with potassium hydroxide digestion and wet mount microscopy. Women that were chlamydia positive were excluded from the study. A urinary human chorionic gonadotropin test was done to rule out pregnancy.

PROTOCOL

This was a triple-blind, placebo-controlled, randomized clinical study. Eligible women were randomized into one of two treatment groups or a control group. The two treatment groups were: 1) *L. acidophilus* NAS strain (H₂O₂ positive; 2 x 10⁹ cfu) vaginal suppositories with placebo (~300 mg [cellulose] Solka-Floc™) oral capsules, and 2) *L. acidophilus* NAS strain (H₂O₂ positive; 2 x 10⁹ cfu) vaginal suppositories with probiotic oral capsules (*L. acidophilus*, NAS strain [5 x 10⁹ cfu]; *Bifidobacterium bifidum*, Malyoth strain [20 x 10⁹ cfu], and *Lactobacillus bulgaricus*, LB-51 strain [5 x 10⁹ cfu]). The control group received placebo (~300 mg Solka-Floc™) vaginally and orally. Oral capsules were self administered daily and vaginal suppositories were self administered three times weekly according to the provided instructions for use. The women were followed for up to six months and seen on a monthly basis and when symptoms were present. The women were requested to abstain from consuming products containing *L. acidophilus* during their participation in the study. As specified by the probiotic

manufacturer, all preparations were kept refrigerated. Financial incentives were not offered for study participants nor were there charges for the study evaluations, laboratory analyses or study products.

Clinical Data Collection

Documented weekly candidal infections, presumed candidal infections, and asymptomatic candidal infections were the three primary clinical outcomes assessed on each study participant. Documented candidal vulvovaginal infections were defined by the presence of erythema and an exudative discharge in association with symptoms of pruritus or pain and the presence of budding yeast or pseudohyphae on KOH digestion or a positive culture for candidal species. In addition, women were seen within forty-eight hours for symptoms of acute vulvovaginitis, or if they were able to come to the clinic, they were encouraged to call the clinic with symptoms. Presumed candidal infections were defined as symptoms of vaginal pruritus or pain with vaginal discharge, which resolved with over-the-counter anticandidal vaginal medication. Asymptomatic colonization with candida was determined on monthly exams during the study period.

Data relating to symptoms of vaginitis, sexual activity, menses and changes in diet or medications were recorded by the attending medical staff during scheduled monthly visits. Vaginal samples were collected at each visit. These samples were subjected to pH measurement and KOH whiff test. Vaginal cultures were collected if these results were equivocal for bacterial vaginosis, yeast infection or trichomoniasis. Candidal vulvovaginal infections were treated with topical terconazole (Terazol[®] 3) 0.8% vaginal cream for three days. No systematic antifungals were used to avoid interfering with the study.

STATISTICAL ANALYSIS

Differences between treatments were evaluated through the analysis of covariance with the number of months in the study as covariate. Several statistical models were evaluated, including covariance models of pooled slopes versus models with a different slope for each treatment group. Single degree of freedom contrasts of adjusted treatment means were also performed. All computations and comparisons were performed in the General Linear Models procedure of SAS (Version 6.12; 1996). Group comparisons at selected time intervals were performed using non-parametric procedures (Mann-Whitney Rank Sum or Kruskal-Wallis ANOVA on Ranks; Sigma Plot 2.0, Jandel Corporation, 1995).

RESULTS

The study was performed from May 1998 to May 2001. During this time 1,090 women presented to the student health center with vaginitis. Seventy-four women who reported a history of RVVC were recruited for the study.

Thirty-four women who met the inclusion criteria were entered into the study. These women had documented candidal vulvovaginitis within the past four months. None of these women was infected with *Gardnerella* species, *Neisseria gonorrhoea*, *Trichomonas* species or chlamydia. Seven women of the original 34 (20%) did not return for any follow up visits. Twenty-seven women returned for follow up visits. (Table 1) Nine women in Treatment #1 returned for follow up visits (0.5-6 months; 34.5 total months). Four women (44%) in this group completed the

entire six months. Eight women in treatment group #2 returned for follow up visits (1-6 months, total 30 months). Three women (38%) in this group completed the entire six-month study. Ten women returned for follow up in the control group (0.5-6 months, total 25.8 months) with two subjects (20%) completing six-month regimen.

The number and rate of documented infections in each group throughout the study period are shown in Table 2 and in Figure 1, respectively. A test of the residuals from the analysis of covariance (ANCOVA), called the Shapiro-Wilks statistic, reveals that the residuals for number of infections and total number of infections plus presumed infections are normally distributed. Table 3 summarizes significance tests for the number of infections.

Twenty-seven subjects participated in the trial for an average of 3.3 months. Since only 33% of the women completed the six-month study, the number of infections was grouped into two 3-month periods (Figure 2). Non-parametric analysis of these data indicate a significant ($P=0.035$) reduction in confirmed infections during months 4-6 among those in the treatment groups.

In the control group, five women discontinued follow up due to frequent candidal vulvovaginitis and one woman discontinued due to a vaginal discharge thought by her to be associated with the suppository. In treatment group 1, one woman discontinued follow up due to reported vaginal irritation and another discontinued due to a mild brown vaginal discharge the morning after applying the suppository. A third woman did not return for clinical follow up. In treatment group 2, one woman dropped out due to a brown vaginal discharge the morning after using the insert. Three other women in this group failed to return for follow up. There was one episode of

bacterial vaginosis in each of the treatment groups. Bacterial vaginosis was not presented by women in the control group.

DISCUSSION

Recurrent urinary tract infections, including candidal vulvovaginitis, are a common problem encountered in primary care and frequently ignored by the medical community.²⁰ Numerous oral and topical therapies for these episodes are available, however the adverse side effects and expense may limit their use.

Premenopausal women present a vaginal microflora that is mostly lactobacilli. Some strains of this genus have certain inherent properties, including adhesive ability and the production of volatile fatty acids, bacteriocins, mucins and H₂O₂. Reid *et al.* recently reported the twice-a-day consumption of *L. rhamnosus* GR-1 and *L. fermentum* RC-14 over a 14-day period may resolved asymptomatic or intermediate bacterial vaginitis.²¹ An earlier study by Hilton *et al.* indicated the consumption of yogurt containing at least 10⁸ cfu/mL of H₂O₂ producing *L. acidophilus* to be effective in reducing the incidence recurrent candidal infections.¹⁷ The application and consumption of selected probiotic strains may provide a unique approach to the reducing the risk of bacterial vaginitis, and may deserve consideration by the medical community as adjunctive agents to protect the urogenital tract against infection.¹¹

Most commercial yogurts contain only unknown strains of *L. bulgaricus* and *S. thermophilus*. The National Yogurt Association recommends that yogurt contain live *L. bulgaricus* and *S. thermophilus* at a concentration of 10⁸ cfu/g. Organism viability and concentrations are not

regulatory statutes, therefore, there is no guarantee that commercially available yogurts today contain adequate number of *L. acidophilus*, which produce H₂O₂ or contain *L. acidophilus* at all.

From a global perspective, there are more than 300 million cases of urogenital infections among women every year. In Western society, these kinds of infections are the primary reason women visit their gynecologist, family practitioner or urologist. Most of these women experience considerable discomfort and these infections may lead to serious medical complications, including extensive health care costs.

Microbial balance may be important in maintaining a healthy urogenital tract. The urogenital microflora of a healthy woman consists of more than 50 different species. The primary uropathogens are *Enterococcus faecalis* and *Escherichia coli*. Most cases of urinary tract infections resolve following appropriate antibiotic therapy. However, the concerns associated with increased antibiotic resistance among these pathogens turn our attention to alternative remedies, including the application of lactobacilli.

This triple-blind, randomized, placebo-controlled trial provides additional evidence that *L. acidophilus* strain NAS (H₂O₂ positive) vaginal inserts properly administered three times per week may significantly decrease candidal infections of this population of women with recurrent vulvovaginitis. The only complaint was an occasional brown discharge following the use of the vaginal insert. This documented complaint was not considered clinically relevant by the attending staff.

The current study among college coeds with RVVC indicates that the intravaginal or oral administration of selected strains of probiotics may significantly reduce the incidence of these recurrent infections. It may be noted that these results may not be applicable to the general population of premenopausal women who suffer from RVVC due to the small sample size of this preliminary study and the demographic nature of the study participants. It is not known whether using the vaginal inserts less frequently would be equally effective or if using these or other selected strains of *L. acidophilus* for shorter periods of time (2-4 weeks) would yield a prolonged or prophylactic effect. However, the work by Reid *et al.* suggests that continual oral consumption or topical application of these strains may be important for reducing the risk of UTI.¹¹ The current study provides additional evidence on the safe use of selected strains of probiotics for a 6-month period to reduce the incidence of urogenital tract infections.

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Table 1. Study group distribution and participation.

Study Group	Number of Women	Average Age (yrs; range)	Total participation (mos)
Control	10	25; 21-34	25.8
Treatment 1	9	27; 19-31	34.5
Treatment 2	8	29; 21-40	30.0

Table 2. Summary of regression parameters for number of documented infections, asymptomatic yeast infections, and total infections plus presumed infections over the 6-month study period.

Study Group	Number of Infections	Slope	Intercept	Correlation Coefficient
Control	10	0.714	- 0.843	0.857
Treatment 1	2	- 0.101	0.611	0.512
Treatment 2	5	0.067	0.373	0.197

Table 3. Significance values for the ANOVA of number of infections:

Number of Infections	P-Value
Treatment differences (intercepts)	0.062
Month effect different from zero	0.004
Month effect differences by treatment	0.001

Figure 1. Total Candidal Infections by Month

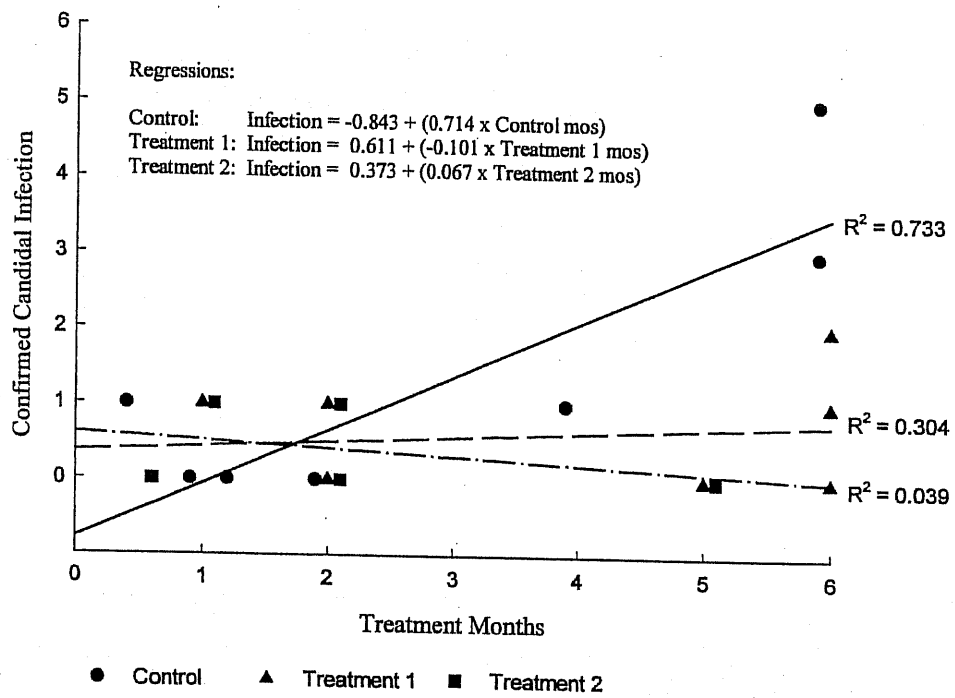
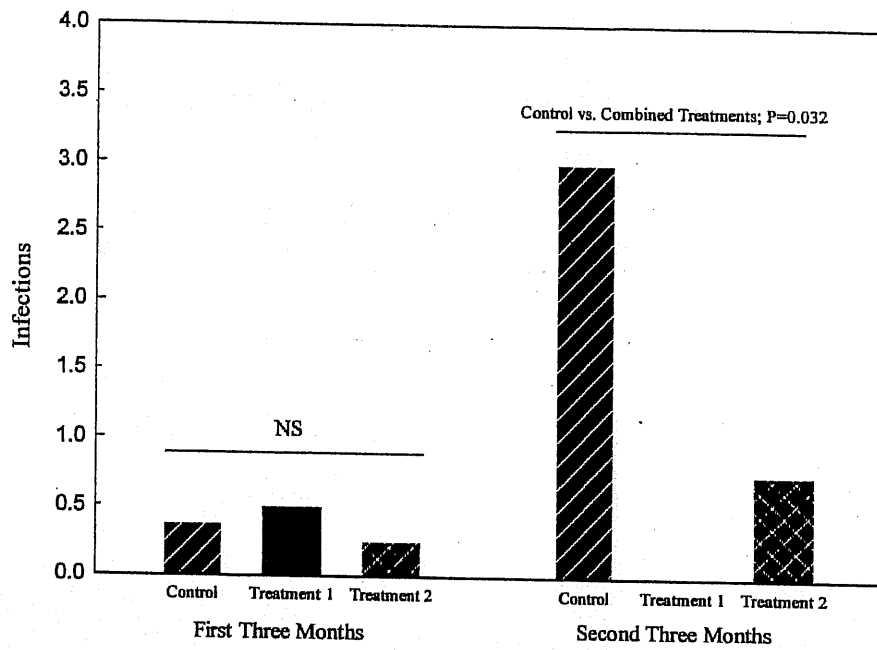


Figure 2. Average 3-Month Incidence of Infections by Study Group



Family Practice Research Presentations (Continued)

Classifications:

- A. Family physicians and fellows primarily in academic medicine
- B. Family physicians primarily in clinical practice
- C. Family practice residents
- D. Medical students
- E. International attendees
- F. Others

The best presentations will receive cash awards of \$1,000, not to exceed seven awards. A maximum of seven runners-up will receive \$250 cash awards. All awards are given at the discretion of the Subcommittee on Family Practice Research Presentations of the Committee on Scientific Program. First-place winners will be acknowledged Saturday afternoon in the Ballroom just after the 3:00 p.m. lecture.

Subcommittee on Family Practice Research Presentations:

Anthony J. Costa, M.D., Chair; Thomas A. Kintanar, M.D.; Renee Miskimmin, M.D.; Eddie Richardson, M.D.; Perry Dickinson, M.D., liaison, Society of Teachers of Family Medicine, and Alicia Vazquez, M.D., liaison, North American Primary Care Research Group

The following schedule gives the title, author(s), presenter (if multiple authors), category, classification and presentation time for each paper. (Schedule subject to change.)

FRIDAY

1:00 - 2:30 p.m.

Knowledge, Attitudes, and Beliefs of Primary Care Physicians on Youth Violence

Shukara Omonuwa, M.D. (presenter) (Category 1, Classification A)

Alzheimer's Disease and Related Dementias Increase the Costs of Comorbidities in Managed Care

Howard Fillit, M.D. (primary author)
 Jerald Hill, Ph.D. (presenter)
 Robert Fulmerman, Ph.D., Sandeep Dattagupta, Ph.D.,
 Vera Masley, MS, John Lloyd, BS (Category 1, Classification F)

Pfizer, Inc. and ISOA provided financial support of this paper.

Adult Inpatient Training for Family Practice Residents: Urban vs. Suburban Settings

Vishal Malpani, M.D., MPH (presenter)
 Roger J. Zornoff, M.D., MPH, Sanjina Malpani, M.D., MPH
 (Category 1, Classification C)

LSUISC Department of Family Medicine provided financial support of this paper.

Practice Dissonance and Satisfaction Among Military Family Physicians

William S. Sykora, M.D. (presenter)
 Glenn Loomis, M.D., Christopher Robinson, Ph.D.
 (Category 1, Classification A)

Uniformed Services AFP provided financial support of this paper.

Alcohol and the Risk of Injury: A Case-crossover Study

Daniel C. Vinson, M.D., MSPH (presenter)
 (Category 1, Classification A)

NIAAA provided financial support of this paper.

Using "Tar Wars" to Prevent Smoking Among Pre-adolescents in Tuskegee

Kevin L. Buford (presenter)
 Ben Rackley, George Rust, M.D., MPH
 (Category 1, Classification D)

TAHEL provided financial support of this paper.

2:30 - 2:45 p.m.

Break

2:45 - 4:15 p.m.

Use of Lactobacillus Acidophilus NAS Strain (H₂O₂ Positive) in the Prevention of Recurrent Vulvovaginal Candidiasis

Julius F. Meets, M.D. (presenter)
 Michelle Famula, M.D., Tom Famula, Ph.D.
 (Category 1, Classification B)

MAFP Foundation, JGAP and Nolren, Inc. provided financial support of this paper.

An Analysis of the Change in Diabetes Control by Utilizing a Full-time Certified Diabetes Educator Nurse in a Primary Care Group Practice

Carl E. Couch, M.D. (primary author)
 Cliff Fullerton, M.D. (presenter)
 Priscilla Hollander, M.D., Pam Sheffield, RN, CDE
 Tracey Gerthoffer, Ph.D., Andrea Ries, PharmD,
 Sally Hill, RN, CDE
 (Category 1, Classification B)

Improving Blood Pressure Control within Amed Healthplan Through Primary Care Provider-focused Interventions

Susan K. Maue, Ph.D. (primary author)
 Marc L. Rivo, M.D., MPH (presenter)
 Stuart Benney, R.Ph., Ph.D., Bruce Weiss, M.D., MPH,
 Eileen W. Farrelly, MPH
 (Category 1, Classification F)

Novartis Pharmaceuticals provided financial support of this paper.

No Extra-fee CME / Family Practice Research Presentations / CME Hours: .25 Prescribed per Paper / Location: Room 305E

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