**ProVate PT-104**

**Clinical Study Report**

**An Open Label, Prospective, Randomized, Controlled, Cross Over Study to Assess Changes in Vaginal Microflora While Using the ProVate Vaginal Device in the Temporary, Non-Surgical Management of Pelvic Organ Prolapse (POP) in Females**

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List of Abbreviations

|  |  |
| --- | --- |
| AE (AEs) | Adverse Event (Adverse Events) |
| ASM | American Society for Microbiology |
| b-hCG | Beta HCG |
| BMI | Body Mass Index |
| BV | Bacterial Vaginosis |
| CDC | The Center for Disease Control and Prevention |
| CFU | Colony Forming Unit |
| CI | Confidence Interval |
| CRA | Clinical Research Associate |
| CRF | Case Report File |
| CRO | Clinical Research Organization  |
| CT | Clinical Trial |
| DIV | Desquamative Inflammatory Vaginitis |
| GBS | Group B Streptococcus |
| HIV | Human Immunodeficiency Virus |
| HPF | High Power Field |
| HR | High Risk |
| HRT | Hormone Replacement Therapy |
| HSV | Genital Herpes  |
| ICF | Informed Consent Form |
| IFU | Instructions For Use |
| Inc./Exc. | Inclusion /Exclusion  |
| IUD | Intra Uterine Device |
| OR | Odds Ratio |
| PID | Pelvic Inflammatory Disease |
| POP | Pelvic Organ Prolapse |
| POP-Q | Pelvic Organ Prolapse – Quantification Scale |
| PP | Per Protocol |
| PT | Preferred Term  |
| SAEs | Severe Adverse Event |
| SOC | System Organ Class  |
| STD’s | Sexually Transmitted Diseases |
| SUI | Stress Urinary Incontinence |
| TSS | Toxic Shock Syndrome |
| UTI | Urinary Tract Infection |
| WHI | Women's Health Initiative  |

# Introduction

## Pelvic Organ Prolapse (POP)

### Definition

Pelvic organ prolapse (POP) is defined as a condition in which vaginal wall support is lost, and various pelvic organs prolapse into the vagina.

### Epidemiology

While minor degrees of POP affect up to 75% of women who have had a vaginal delivery[[1]](#endnote-1), symptomatic POP with descent beyond the hymen affects 3% to 6% of the population[[2]](#endnote-2). The most commonly reported figure is that ~3.5 million US women (range 1.36-5.33) currently suffer from symptomatic POP[[3]](#endnote-3). Approximately10% of the currently impacted population will require surgery to correct the prolapse, of which ~30% will require at least another 1-5 operations[[4]](#endnote-4). Every year, 210,000 - 300,000[[5]](#endnote-5) women undergo surgical interventions for the disorder.

In a study published in 2001, current demand for consultations due to POP was calculated, and growth in demand for services to care for POP was extrapolated. It was estimated that within 30 years, demand for services will grow by 45%, while the population will only grow by 22%[[6]](#endnote-6).

 POP is very much ethnic dependent, where highest prevalence is among white women and lowest among African American women. Whitcomb et al. evaluated prevalence of POP in a diverse, population-based cohort[[7]](#endnote-7) (n= 2270 women) and found that compared with African-American women, Latina and white women had four (4) to five (5) times higher risk of symptomatic prolapse, and white women had 1.4-fold higher risk of objective prolapse with leading edge of prolapse at or beyond the hymen. Sewel et al.[[8]](#endnote-8) screened 167 women, finding that sixty-seven (67%) percent of Asian American patients had stage 2 or higher prolapse as compared to 26% of African American and 28% of white patients. Multiple logistic regressions showed that Asian American ethnicity independently correlated with higher rates of pelvic organ prolapse. Hendrix et al.[[9]](#endnote-9) conducted analysis on women from the Women's Health Initiative Hormone Replacement Therapy Clinical Trial (WHI HRT CT) (n=27,342 women). They noted that the risk for prolapse differs between ethnic groups, and that African American women demonstrated the lowest risk for prolapse. Hispanic women had the highest risk for uterine prolapse. In a different study it was shown that African Americans were less likely (HR 0.53, 0.40–0.71) to develop Grade 2/3 POP compared to whites.[[10]](#endnote-10) In another study[[11]](#endnote-11) it was shown that African American women are significantly less likely to report symptomatic prolapse, compared with white women (OR 0.4, 95%CI 0.2-0.8), hence will probably seek treatment less frequently.

In other words, the findings of increased symptoms in white and Latina women, despite similar POP-Q stages, may suggest that the racial disparity in prevalence of subjective prolapse is in part attributable to cultural attitudes toward the symptomatology of prolapse or its reporting. There is a very high non-disclosure rate among women with POP in general, and the lower rate of POP in African American may also be due to tendency not to complain or disclose presence of a prolapse.

This point of the rather low occurrence of symptomatic POP within the African-American population, and difficulty in recruiting African American women with symptomatic POP was raised by Dunivan et al[[12]](#endnote-12), who looked into the data of 588 non-Hispanic white, Hispanic, and Native American women, but were only able to trace 9 African American women.

During interviewing of investigators for the PT-104 study, and during site selection, many of the clinics stated that African American women with POP of stages ≥2 are infrequently seen, that they hardly have any African American patients with a symptomatic prolapse which is also treated, and that these women are less likely to look for any treatment. Therefore, chances of recruiting African American women who are using a ring pessary for POP are rather low, even if recruitment is done outside of the clinics, by public advertisement.

### Symptoms and Complaints

Symptoms of POP may vary from being completely absent to a combination of various complaints which may be very bothering and influence everyday life. Most prevalent POP symptoms include

* Feeling of pelvic heaviness
* Bulging out of the vagina
* Vaginal discomfort / pains
* Pelvic and/or lower back pain
* Recurrent bladder infections
* Voiding difficulties up to retention
* Difficulty emptying the bowels
* Difficulty inserting or keeping a tampon in place
* Vaginal-cervical mucosa hypertrophy, excoriation, ulceration, and bleeding

### Management Options

Within the ~3.5 million US women with POP who are considered to have symptomatic POP, there are 4 available management options:

1. *Expectant* – women may consult regarding their problem, but will prefer to defer any management due to various reasons.
2. *Reconstructive surgical management* – various operations, by various routes, with or without implants. The purpose of the surgery is to correct the anatomy as well as provide better bowel, bladder and vaginal function.

Prolapse repair can be done trans-vaginally, abdominally, laparoscopically and/or robotically. Correcting all support defects is paramount in the surgical approach to POP. Approximately 11% of women will have surgery for POP prior to 80 years of age. Unfortunately, nearly 30% of these women will need another surgery due to failure or recurrence of prolapse (though some researchers demonstrate up to a 56% reoperation rate[[13]](#endnote-13),[[14]](#endnote-14)) or for treatment of another pelvic floor problem4. In 40% of cases, there is failure to treat symptoms (even though anatomy may be restored).

Use of permanent mesh that is placed vaginally may improve initial vaginal support but is associated with complications, such as persistent pain and tissue erosion, some of which may require additional surgeries to correct. Recent literature has shown that there is no benefit in using mesh grafts in POP surgical repairs for greater durability, results do not sufficiently outweigh the risks of undesirable adverse consequences, and that native tissue and vaginal mesh surgery had similar 5 year risks for recurrent prolapse[[15]](#endnote-15). Limited research to date indicates that women who are older, smoke, are diabetic, or have had a hysterectomy are at higher risk for these more common complications. In 2008, and again in 2011, the FDA issued warnings that synthetic mesh repair is associated with the above serious complications.

1. *Non-surgical management*
	1. *Pelvic Floor Physiotherapy/Kegel exercises*

Pelvic floor physiotherapy is generally regarded as acceptable for stages 1 and milder stage 2 POP[[16]](#endnote-16). It is therefore considered a way for relieving some POP symptoms, but it is no longer considered a management option for more severe cases.

* 1. *Vaginal pessaries*

A pessary is a device inserted into the vagina to support the walls and related pelvic organs[[17]](#endnote-17). Modern pessaries are made of hypoallergenic silicone, rubber, or pliable plastic and are indicated for all pelvic prolapse stages[[18]](#endnote-18).

Pessaries are considered to be a relatively safe method of managing pelvic organ prolapse without serious side effects[[19]](#endnote-19),[[20]](#endnote-20),[[21]](#endnote-21).

## Existing Vaginal Pessaries

Most women with POP will only require non-invasive management[[22]](#endnote-22). Vaginal pessaries are the only proven and well-studied noninvasive means of managing POP. A pessary is a device inserted into the vagina to support the walls and related pelvic organs. Modern pessaries are made of hypoallergenic silicone, rubber, or pliable plastic and are indicated for all pelvic prolapse stages[[23]](#endnote-23). 77% of gynecologists in the US prescribe pessaries as first-line therapy for women with POP[[24]](#endnote-24), although training in pessary use is limited[[25]](#endnote-25). Similarly in the UK, 86.7% of obstetricians and gynecologists surveyed, prescribed pessaries in the management of POP[[26]](#endnote-26). Pessaries are experiencing resurgence in popularity following the problems experienced with surgical solutions (high symptomatic recurrence rate and morbidity with the trans-vaginal mesh implants), and are now again viewed as an option for the management of prolapse for women in any age group. Reports suggest that 76% of women can be successfully fitted at the follow-up visit (average 1.5 (range 1-4) pessaries). Success or failure will depend on appropriate pessary selection, patient characteristics, provider training and experience, thorough counselling, as well as the achievement of an adequate fit and patient satisfaction.

Overall, it appears that 56% to 73% of women with symptomatic pelvic organ prolapse can be fitted successfully with a pessary[[27]](#endnote-27). The pessary size used should be individually fitted to each patient. According to the data provided in the current clinical literature, trial and error is still the only way to determine the proper size of the pessary; however, an approximation of the size can be made by measuring the width and length of the vagina with the fingers of the examining hand. This initial measurement will usually be accurate to within one (1) or two (2) sizes of the appropriate pessary.

A well-fitted pessary should fill the vagina from side to side, with the clinician’s finger able to easily pass between the pessary and the pelvic sidewall around the entire circumference of the pessary. When the pessary is properly placed in the upper vagina, the patient should be unaware of its presence. The patient is asked to stand and move about to see if the pessary remains in place. If the patient becomes aware of the pessary at the introitus, the next larger size should be tried. If the patient is aware of tightness, pressure, discomfort or pain, fitting with the next smaller size pessary should be attempted. The patient is then often asked to perform a set of typical activities in the office such as walking, fitting, climbing stairs, bending and sing on the toilet.

The average number of pessaries tried during a successful pessary fitting is 2-3, typically in a single session. However, up to two (2) follow-up fitting sessions have been reported prior to successful fitting. At such follow-up visits, the pessary size is increased in patients whose pessary slipped and is decreased in patients experiencing pelvic discomfort or pressure on pelvic organs.

It is accepted that most of the prolapse cases which can be managed by pessaries, may be managed by ring pessaries, with or without internal support, and this is the most commonly used configuration of pessary[[28]](#endnote-28). Vaginal ring pessaries are known to the medical literature for many centuries.

Pessaries have been in the market for many decades, and they are known to be relatively safe for use, involved with minor complications, most of them anticipated.

Vaginal wall trauma (a term which includes erosions, abrasions, lacerations, ulcerations, etc.) is the most frequently reported complication of a pessary[[29]](#endnote-29), presenting as foul odor, purulent discharge, irregular blood stained discharge, and increased vaginal fluid. It occurs in ~19.3% of pessary users[[30]](#endnote-30) (range 3-48.2%[[31]](#endnote-31),[[32]](#endnote-32)), and believed to be caused by pressure on vaginal walls or by the initial phase of device usage (accommodation period). Pain and spotting are also seen very often among pessary users. Reports of pain in pessary users ranges 6.9-41%[[33]](#endnote-33),31, with discomfort only rarely being reported as an adverse event. The reported rate of spotting is quite similar, in the range of 6.9-47%[[34]](#endnote-34),34. Other anticipated AEs, such as urogenital infections (vaginal and urinary) are reported in the range of 13.5%-25.5%[[35]](#endnote-35),[[36]](#endnote-36) and De Novo stress urinary incontinence and constipation are also seen, however in lower rates.

## Vaginal Microflora

### The Normal Vaginal Microflora

Humans serve as hosts to co-evolving microbes residing in highly plethoric communities. Indeed, microbiota is present from the time of birth and changes throughout life.

The vagina of healthy premenopausal women is dominated by lactobacilli, which play an important role in protecting the host from urogenital infections. Furthermore, it is widely recognized that the microbial balance between lactobacilli as the dominating flora and others, mainly gram-negative anaerobes, may predominate.

Anatomically, the female genital tract is formed of a chain of cavities. These structures allow for the menstrual outflow, sexual intercourse, and the delivery of babies, however also enable the entrance of potentially pathogenic bacteria. The vaginal microflora undoubtedly presents one of the most important defense mechanisms of the human genital tract and the reproductive function by maintaining the healthy environment and preventing the proliferation of microorganisms that are foreign to the vagina.

Accordingly, similar to other anatomical body orifices, no two (2) women have identical vaginal microbiomes. Moreover, the microbiomes of individuals are subject to many influences which may dramatically alter the microbiome character and composition. Such influences may include the role of reproductive hormones resulting in dramatic changes during puberty and menopause. Similarly, profound alterations in the microbiome are seen during symptomatic vaginal infections (e.g. Trichomoniasis and bacterial vaginosis). Even in healthy women, day to day variations in the vaginal microbiome may follow behavioral interventions such as douching, stress and sexual intercourse. During menses, bacterial microbiota alters in the presence of vaginal blood only to revert to the premenstrual composition with cessation of menses.

For the majority of women during the years of reproductive health, the vaginal microbiome is dominated by several Lactobacillus[[37]](#endnote-37) species, most commonly *L. jensenii, L. crispatus, L. acidophilus* and *L. Doderlein*, which elaborate bacterocins and contribute organic acids dominated by lactic acids to achieve pH stability at a range of pH 3.8 – 4.5. In these healthy, asymptomatic (“normal”) women, vaginal health is indicated by a vaginal pH of 3.8 – 4.5 with transient increases in pH immediately following unprotected coitus and throughout the menses.

During the postmenopausal period, the vaginal epithelium becomes very thin and the content of glycogen decreases, leading to changes in vaginal pH and in microflora itself. During post menopause, Lactobacilli are reduced, and microorganism such as Escherichia Coli, Enterobactericeae, Entrococci, *Staphylococcus aureus*, and other facultative microorganism, may be present in the vaginal flora[[38]](#endnote-38) as well.

On some occasions, when there are clinical symptoms such as vaginal purulent discharge accompanied by vaginal microflora imbalance, the woman is diagnosed as suffering from bacterial vaginosis.

### Vaginal Microflora in Different Ethnicities

Women of European ancestry are more likely to harbor a Lactobacillus-dominated microbiome, whereas African American women are more likely to exhibit a diverse microbial profile and are also twice as likely to be diagnosed with bacterial vaginosis. This was discussed in several studies[[39]](#endnote-39); however the exact clinical importance of this finding is not clear as this difference does not necessarily signify a pathologic finding and is found in healthy asymptomatic women where treatment is not warranted in the absence of complaints.

### Vaginal Microflora - Normal Fluctuations

Vaginal microflora changes frequently, even on a daily basis, due to various reasons; however, this is not necessarily indicative of or followed by vaginal infection. Bacterial communities within the vagina are unstable, and variations in the composition of vaginal bacterial communities are very often seen, even on a daily basis. Such fluctuations in vaginal microflora are not necessarily associated with overt vaginal infection or with any accompanied symptom or sign.

Deviations from vaginal floral stability correlate with many factors, including:

* Original bacterial community composition, history of previous BV and other STD’s[[40]](#endnote-40)
* Race/ethnicity[[41]](#endnote-41),[[42]](#endnote-42)
* Menstruation and time in the menstrual cycle[[43]](#endnote-43),[[44]](#endnote-44) (in menstruating women),
* Sexual activity & number of partners[[45]](#endnote-45), receiving oral sex, anal sex[[46]](#endnote-46), gender of sexual partner[[47]](#endnote-47)
* Spermicide use & condom use18
* Medications – systemic or vaginal medications,(including antibiotics, hormonal contraceptives[[48]](#endnote-48))
* Various perineal and vaginal cosmetics[[49]](#endnote-49)
* Various behavioral factors such as perineal cleaning, douching[[50]](#endnote-50), etc.

**In most cases these fluctuations are transient, but may occur very often, hence the question regarding the “real” or “normal” vaginal flora.**

Bacterial Vaginosis (BV) is characterized by dramatic changes in the vaginal ecosystem. This may be symptomatic or asymptomatic, and women without evidence of vaginal infection (e.g. abnormal vaginal discharge, vulvar/vaginal itching and irritation, abnormal odor, vaginal inflammation, and vaginal tenderness) may exhibit transient microbiological changes in their flora, and are NOT diagnosed with vaginal infection.

Schwebke et al36 (USA) prospectively followed 51 women completers for 6 weeks, by using diaries and self-obtained vaginal smears to correlate behaviors with changes in vaginal flora. Approximately 50% of the study population was African American, 1.7% Asian and the rest were Caucasian. In this study, the majority of the women (78%) had significant, although transient, vaginal flora changes, as defined by the Nugent’s score. Only a minority of women (22%) maintained a “normal” lactobacillus-predominant flora during the entire study, as defined by consistency in the Nugent’s score (Nugent’s[[51]](#endnote-51)) at study visits. Transient fluctuations in vaginal flora were common and often marked. An important message from this study is that factors associated with instability of the flora are similar to those epidemiologically associated with BV. Since Incidence of symptomatic BV is rather low compared to incidence of vaginal flora instability/fluctuations, relying only on vaginal lab results may lead to over-diagnosis of vaginal flora abnormalities, which should otherwise be attributed to daily fluctuations in otherwise healthy women. Brotman et al19 (USA) conducted a prospective longitudinal study to assess changes in vaginal microbiota. Thirty-nine (39) women self-collected vaginal specimens twice-weekly for 16 weeks. Rapid fluctuation of vaginal microbiota was observed in 226 transitions to BV or spontaneous remission. Of the 113 observed remissions, all but one (1) were spontaneous (not as a result of antibiotic treatment). Duration of BV was often short: 51% of the episodes lasted only one (1) sample interval (three (3) days). This study confirms again that women may have short episodes of disruption of vaginal microbiota (that spontaneously resolve), but may present at times a laboratory diagnosis of BV which is totally asymptomatic in the woman, and does not require treatment. Gajer et al[[52]](#endnote-52) (USA) prospectively followed 32 reproductive age women over a 16-week period. Samples were taken twice weekly. Analysis of the results revealed that some bacterial communities change markedly over short time periods, whereas others are relatively stable. Deviation from stability correlates with time in the menstrual cycle, bacterial community composition and sexual activity

Ravel et al15 (USA) reported on temporal dynamics of 25 vaginal communities over a 10 week period using samples collected daily from women who were diagnosed with symptomatic BV (15 women), asymptomatic BV (6 women), and women who did not have BV (4 women). The vaginal microbiota of women who did not have symptomatic or asymptomatic BV was consistently dominated by Lactobacillus spp. or Bifidobacterium. The investigators point out that BV is a common gynecologic diagnosis characterized by dysbiosis of the vaginal microbiota. However, though sometimes accompanied by vaginal symptoms such as odor and discharge, it is largely asymptomatic. The key messages from this study are that fluctuations in vaginal bacterial communities are common, and that women may have short episodes of BV that spontaneously resolve without antibiotic therapy. As guided by the CDC, and by other clinical organizations outside the USA, these conditions do not necessitate any treatment, unless accompanied by specific vaginal complaints and / or symptoms such as thrush, pain, discomfort, infectious discharge, smell, etc.

**The conclusion from these studies is that microflora changes by itself will not lead to the diagnosis of BV, and this diagnosis is valid only with the combination of clinical symptoms accompanied by changes in the microflora.**

### Vaginal Infections

Symptoms corresponding with vaginal infection (vaginitis) include abnormal vaginal discharge, vulvar/vaginal itching and irritation, and/or an abnormal vaginal odor. On examination there may be signs of vaginal inflammation, tenderness, abnormal discharge and even foul odor.

The most common causes of symptomatic vaginitis are bacterial vaginosis (presence of *Gardnerella vaginalis* and reduction in the amount of Lactobacillus spp (15% to 50%[[53]](#endnote-53),[[54]](#endnote-54))), vulvovaginal candidiasis typically due to *Yeasts* infection (~33%[[55]](#endnote-55)), and Trichomoniasis due to *Trichomonas Vaginalis* infection (4-20%[[56]](#endnote-56),[[57]](#endnote-57),[[58]](#endnote-58)).

**Vaginitis – specific types of vaginitis & indications for treatment**

**Bacterial Vaginosis (BV)** Bacterial Vaginosis (BV) is characterized by dramatic changes in the vaginal ecosystem - significant reduction in lactobacilli and increase in Gardnerella Vaginalis. This may be symptomatic or asymptomatic, and women without evidence of vaginal infection may exhibit transient microbiological changes in their flora, and are NOT diagnosed with vaginal infection.

For symptomatic cases, symptoms include vaginal discharge, where BV is the most common cause of vaginal discharge in women of childbearing age, accounting for 40 to 50 percent of cases[[59]](#endnote-59). The absence of inflammation is the basis for the term "vaginosis" rather than "vaginitis."

Worldwide, BV is common among women of reproductive age, with variations according to the population studied. Bacterial vaginosis represents a complex change in the vaginal flora characterized by a reduction in concentration of the normally dominant hydrogen-peroxide producing lactobacilli and an increase in concentration of other organisms, especially anaerobic gram negative rods, with the most prominent being *Gardnerella vaginalis*.

The diagnosis of bacterial vaginosis is based on clinical complaints, vaginal findings and laboratory tests Gram's stain of vaginal discharge is the gold standard for laboratory diagnosis of BV, using the Nugent’s score.

The Center for Disease Control and Prevention (CDC), in its 2015 guidelines for treating Bacterial vaginosis[[60]](#endnote-60) states that

* **“Treatment [in non-pregnant women] is recommended for women with symptoms. The established benefits of therapy in non-pregnant women are to relieve vaginal symptoms and signs of infection.** Other potential benefits to treatment include reduction in the risk for acquiring C. trachomatis, N. gonorrhoeae, T. vaginalis, HIV, and herpes simplex type 2”.
* In pregnant women, BV may increase the likelihood of preterm birth, and therefore treatment may be recommended for all symptomatic pregnant women.

Sobel JD in UpToDate (March 2017)[[61]](#endnote-61) states that **“Approximately 50 to 75 percent of women with BV are asymptomatic. Treatment is indicated for relief of symptoms in women with symptomatic infection and to prevent postoperative infection in those with asymptomatic infection prior to abortion or hysterectomy.** Some experts support the concept of treating all women with BV regardless of presence or absence of symptoms; however, we agree with the United States Centers for Disease Control and Prevention (CDC) recommendations to not treat asymptomatic women”.

The European Guidelines (IUSTI/WHO) on Treatment of Vaginal Discharge 2011[[62]](#endnote-62) discusses the indications for treatment of BV. **The indication for treatment in non-pregnant women are symptomatology and in some surgical procedures**. The report also states an optional possibility to treat some cases where microscopy is positive for BV but except for discharge there are no other symptoms - as this may be beneficial in reducing the discharge.

UK National Guideline for the management of Bacterial Vaginosis 2012 (by the British Association for Sexual Health and HIV)[[63]](#endnote-63) recommends treating patients with BV **– if they are symptomatic, are undergoing some surgical procedures**, and may elect to take treatment as they may report a beneficial change in their discharge following treatment.

Koumans et al[[64]](#endnote-64) have looked at the indications for therapy and treatment recommendations both in pregnant and non-pregnant women in a literature search over 26 years. In the past, the goal of therapy for non-pregnant women with BV has been resolution of BV in the vagina. The present detailed review has shown the limitations in currently recommended therapies, as **in most women the symptoms of BV resolve on their own without intervention[[65]](#endnote-65)**, with worst-case relapse rates 1 month after therapy of up to 56%. More than that, the “cure rates” of women given placebo (22%–25% after 1 week) were also high. This raises several questions about the specificity and stability of the diagnosis of BV over time. When diagnosis is by Gram's stain, some women with altered flora will restore a lactobacillus-predominant flora without therapy. There did not seem to be any differences in efficacy among trials that enrolled only symptomatic women and trials that enrolled women regardless of symptoms.

Sobel et al[[66]](#endnote-66) mentioned that even for recurrent BV infections – management strategies are not standardized and indications for treatment are not final.

**Candidiasis**

Vulvovaginal candidiasis refers to a disorder characterized by signs and symptoms of vulvovaginal inflammation in the presence of Candida species. It is the second most common cause of vaginitis symptoms (after bacterial vaginosis) and accounts for approximately one third of vaginitis cases[[67]](#endnote-67). It is generally not considered an opportunistic infection, and is not considered a sexually transmitted disease. Candida species can be identified in the lower genital tract in 10 to 20 percent of healthy women in the reproductive age group, and in 6 to 7 percent of menopausal women.

Identification of vulvovaginal Candida is not necessarily indicative of candidal disease, as the diagnosis of vulvo-vaginitis also requires the presence of vulvovaginal inflammation. Treatment is indicated for relief of symptoms. 10-20% of reproductive age women who harbor Candida species are asymptomatic, thus are NOT diagnosed with vaginal candidiasis; These women do not require therapy[[68]](#endnote-68).

***Staphylococcus aureus***

There is only little evidence in the literature for vaginitis (with both symptoms and signs) caused by this bacterium (unless part of TSS which is a systemic condition).

### Vaginal Devices and Microflora Changes - Data from the Literature

There are many instances where devices are used vaginally, yet the clinically manifested vaginitis rate is rather low. The most commonly used long-term vaginal device is the intra-vaginal pessary for POP, followed by vaginal contraceptive rings. Vaginal contraceptive rings have a definite lifetime of 21-28 days in which they remain within the vagina. However, there is no definite or agreed-upon length of use for POP pessaries. The question of whether vaginal devices cause significant changes in vaginal microflora, and whether such devices may increase the rate of vaginal infection, has been the subject of many studies:

* De Seta et al.[[69]](#endnote-69) compared the effects of two (2) hormonal contraception methods – oral tablets and vaginal ring - on vaginal milieu. They examined vaginal pH; quantification of leukocytes, lactobacilli, Candida and cocci on saline microscopy fluid; Gram stain with Nugent score, and the presence of vaginal infection [culture for *Trichomonas vaginalis*, *Candida* albicans and nonalbicans Candida, and Group B Streptococcus (GBS)]. In a prospective comparative study on 60 women over 1 year, there was a little change of vaginal milieu in both groups. However, they have noted an increase of lactobacilli in the group of subjects who used the vaginal ring (interpreted as protective in terms of prevention of vaginal imbalance/infection) as opposed to an increase of GBS in the oral tablets users group.
* Del Priore et al.[[70]](#endnote-70) conducted a pilot safety and tolerability study of a non-hormonal vaginal contraceptive ring (Ovaprene, Poly-Med Inc). Following one (1) cycle use, semi quantitative cultures yielded no significant changes in vaginal flora, compared with baseline.
* North and Oldham[[71]](#endnote-71) examined microbiological safety of the Softcup® menstrual cup (intended for the intra-vaginal collection of menstrual blood). Vaginal flora was examined after 1, 2, and 3 months of cup use. Women were then followed for up to 3 additional months after returning to their normal method of menstrual protection (pads or tampons). Vaginal flora samples (Staphylococcus aureus, group B Streptococcus, Enterococcus, Escherichia coli, Candida spp., Gardnerella vaginalis, Bacteroides*spp.,*Lactobacillus) were collected. At baseline, 91% of the women harbored lactobacilli, *G. vaginalis*, group B Streptococcus, Enterococcus, S. aureus, *E. coli*, yeast, and Bacteroides spp. at frequencies considered normal and consistent with the selection of genital infection-free women. Use of the cup over three (3) successive menstrual cycles had no effect on vaginal colonization by S. aureus, did not lead to increased colonization by microorganisms associated with bacterial vaginosis (*G. vaginalis* and Bacteroides spp.), vulvo-vaginitis (Candida and other yeast), or urinary tract infections (E. coli). Also, vaginal Lactobacillus (normal vaginal flora) was maintained at normal levels before, during, and after use of the cup.
* Vaginal microflora studies conducted at various times during the menstrual cycle have shown menstrual tampons to have no significant effect on the qualitative or quantitative changes in either the aerobic or anaerobic organisms which occur normally during menses[[72]](#endnote-72). Chase et al.[[73]](#endnote-73),[[74]](#endnote-74) examined possible changes in vaginal microflora while usage of 2 different menstrual tampons (“Winged” Apertured Film Cover and a Commercial Tampon with a Nonwoven Fleece Cover), and showed that there was also no difference between different kinds of tampons.
* Lea’s Shield is an intra-vaginal contraception device, approved by FDA. In a 2005 published study[[75]](#endnote-75), the authors report on 30 subjects who used the product for eight (8) weeks. Although average colony counts for enterococcus, Escherichia coli and anaerobic gram-negative rods increased during product use, no clinical diagnoses of infection were made. The authors conclude that these changes in vaginal microflora do not appear to correlate with clinical infections which inherently require the presences of symptoms.
* Collins et al[[76]](#endnote-76) conducted a comparative clinical study to evaluate the differences in vaginal culture, microscopy and gram stain and to explain pessary-related bothersome vaginal discharge, between 50 post-menopausal women using pessaries (“established users”), and 50 women who were prior to their first pessary fitting (“new users”). Women using pessaries in the study were more likely to be bothered by discharge, and were more likely to show microscopic evidence of vaginal inflammation. Clinical evaluations showed that these changes developed within 2 weeks of pessary use for new users. Culture results were analyzed for bacteria considered physiologic (Lactobacilli), and those considered pathogenic (Gardnerella, Bacteroides, Prevotella, Porphyromonas, and Mobiluncus spp). There were no significant differences in the prevalence of any of the above bacteria between any of the time points analyzed during the 6 month evaluation period. The Authors concluded that discharge in post-menopausal pessary users is mainly due to Desquamative Inflammatory Vaginitis (DIV), not an infectious process.
* In a study by Gorti et al5, changing of pessaries (e.g., removal and cleaning) occurred at any time between 3-12 months, and the authors concluded that a 6-12 months changing interval was safe. The authors could not demonstrate a difference in the rate of total AEs when the pessary was replaced every 3 months or between 3-6 months. However, there was an unexplained decrease in the rate of total AEs when pessaries were replaced every 6-12 months.
* During a study of the TIPI/Impressa intra-vaginal stress urinary incontinence device (K131198) (TIPI 002 study, Ziv et al[[77]](#endnote-77).), 60 participants used the cleared version of the device for 8 hour daily, for 28 days, not necessarily every day. There were 2 cases of clinical yeast infection during the study (one of them following ingestion of antibiotics for Follicular Tonsillitis), and 4 cases of asymptomatic candidiasis detected at the end of the study (according to the protocol). As it is estimated that asymptomatic vaginal yeast infections may be found in 15-25% of healthy women[[78]](#endnote-78), this figure of 4 participants (4/60=6.6%) with post study asymptomatic Candidiasis, stands within the normal published rate of vaginal candidiasis.
* In a USA post-market study (Farage et al.[[79]](#endnote-79)), the same model of the TIPI Device was used for 12 hours in every 24 hours period, and there were no clinically significant changes from baseline with regard to vaginal pH at the end of the study. Women with higher and lower pH values at baseline shifted toward the baseline mean of 4.5 after device usage.
* In cases of cervical incompetence during pregnancy, either cervical suture (Cerclage) or cervical pessary may be applied (sometimes both), remaining within the vagina for many weeks. In a Cochrane Database Systematic Review[[80]](#endnote-80), it was shown that cervical cerclage was associated with higher rate of vaginal discharge, as compared with expectant management only, but there is no mentioning of vaginal infection. Kessler et al[[81]](#endnote-81) have compared vaginal cultures taken before and after putting a cervical suture in 25 patients, and found no significant difference between the positive results before and after the procedure, but also when comparing the results to randomly selected pregnant women who did not need a cerclage.

As may be noted from this section – there are many vaginal devices in the market but there is no compelling evidence that such devices cause significant changes in vaginal microflora while in use.

## The ProVate Device

The ProVate Vaginal Support (also termed the ProVate Device) is a vaginal ring pessary intended for the conservative nonsurgical, temporary management of Pelvic Organ Prolapse (POP) in females. The ProVate is a disposable, single use device intended to be worn (remain in the vagina) for up to seven (7) days. The ProVate is intended for prescription use. Size fitting, patient training and routine follow up are performed by the health care professional.

When deployed within the vagina, the ProVate Device performs in a similar manner to existing ring pessaries, e.g. by providing mechanical support to the descending organs. Specific features for the ProVate Device are being a single use device (disposable), provided with an applicator and a removal string. These design features are intended to overcome the specific shortcomings of existing pessaries and enhance usage:

* Allowing the user to insert and remove the ProVate Device by herself in her home environment with minimal self-touch and avoiding the need for removal and cleaning by a healthcare professional at the clinic.
* **The ProVate Device is inserted and removed in small dimensions, making insertion and removal more comfortable. The ProVate Device is only expanded into its ring shape when inside the vagina in the target anatomical area to provide the necessary support.

Figure 1-The ProVate Device with its various components

The ProVate Device is provided ready for use, compressed within an applicator. Its main 2 components are the bi-modal Ring Support (which may be in its compact within the applicator and have a ring shape when deployed) and the Applicator. Deployment of the ring Support happens while it is already within the vagina to minimize pain and discomfort often associated with the insertion of vaginal pessaries. At the end of use, when the removal string is pulled by the user, the ring Support collapses back into its small compact dimensions for comfortable removal.

The ProVate Device is made of a flexible polymeric skeleton covered by a soft elastomer. Once deployed within the vagina, as with other ting pessaries, the ProVate Support distends lateral vaginal walls aside, mechanically prevents cervical/vault descent, and with its central piece – blocks further descent of the anterior/posterior walls within the hollow of the ring.

The ProVate Device is provided in 6 sizes, ranging from 61mm to 91mm, to accommodate various vaginal dimensions. The Device comes ready for use in small dimensions (compact mode) within a disposable applicator. The applicator allows for a smooth and comfortable insertion into the vagina, resembling the concept of insertion of a regular menstrual tampon. When the plunger of the applicator is depressed, the Device becomes fully deployed, restoring its predefined size (deployed mode), and separates from the applicator which is then removed by the user from the vagina for disposal.

The Device may be left in place within the vagina for up to 7 days. Following usage of up to 7 days, the user pulls the removal string, and the Device collapses into its narrow pre-insertion dimensions (compact mode) for an easy and comfortable removal out of the vagina for disposal

As may be noted in Figures 1 and 2, the Support comes ready for use within the Applicator (Figure 1 left) where the Support is in its compact mode. Once the Applicator Plunger is pressed, the Support deploys into the ring shape (Figure 1 low right). Then, the Support separates from the Applicator which is discarded by the user. By the end of use – the user pulls the removal string, and the Device becomes compact again, and slides out of the vagina for disposal.

Figure 2 below shows the ProVate Device in different configurations, compacted and deployed, with and without the Applicator, within and outside the body.

|  |  |
| --- | --- |
|  | 07.tif |
| **Figure 2b-The ProVate Support, within its Applicator, inserted intra-vaginally** | **Figure 2a The ProVate Support, in its compacted mode within the Applicator** |
| 09.tif |  |
| **Figure 2d-The ProVate Support in its deployed (circular ring shape) mode, during use** | **Figure 2c-The ProVate Support in its narrow compact mode, without the Applicator** |
|  |
| **Figure 3e-The ProVate Support in its narrow compact mode, pulled out of the vagina for disposal** |

Figure 2-The ProVate Device in different configurations

# Materials & Methods

## The PT-104 Study

### Study Objective

The objective of the study was to confirm that the ProVate Device does not alter vaginal microflora in a clinically significant manner, as compared to a Control (commercially available vaginal ring pessary).

### The Study Endpoints

1. **The Primary Endpoint** is based on a failure criteria**:** a subject is a failure if there is a significant change in vaginal microflora (i.e., significant meaningful change in Lactobacillus spp*.*, *Gardnerella vaginalis*, Candida morphotypes, or*Staphylococcus aureus* levels from baseline), where the significant change is defined, according to the common clinical practice, as:
2. Nugent score ≥ 7, or > 1 scale unit increase in *Staphylococcus aureus* or Candida morphotype; or;
3. Vaginal symptoms that are bothersome to the subject, or;
4. Vaginal symptoms that require treatment.
5. **The Secondary Endpoints** are:
6. Proportion of subjects with changes in microbial counts following device usage, compared to baseline, greater than 1 unit scale (> 1 unit scale) in any of the 4 study microorganisms.
7. Proportion of patients who have a Nugent score ≥ 7 for Lactobacillus spp*.* and *Gardnerella vaginalis* following device usage.
8. Proportion of patients who have a >1 scale unit increase in *Staphylococcus aureus* following device usage.
9. Proportion of subjects who have a >1 scale unit increase in Candida morphotype following device usage.
10. Proportion of subjects who have vaginal symptoms are bothersome following device usage.
11. Proportion of subjects who have vaginal symptoms that require treatment following device usage.

### Study Population

The study population included females aged 21 to 80 years who are in good general health and suffer from POP (POP-Q stages 2-4). 73 subjects were randomized in a 1:1 ratio to groups A and B. Premenopausal women were enrolled only if their menstruation cycles within six (6) months prior to the study were regular and lasted between 26-40 days. The study population consisted of women who have previously used any ring vaginal pessary.

The study took place in 7 sites; six (6) sites were in the US and one (1) site in Israel. Over 60% of study subjects were recruited in the US sites.

### Study Design

The study was designed as a multi-center, open label, prospective, randomized, controlled, statistically powered (non-inferiority), cross over, home-use study, conducted in seven (7) sites in the US and in Israel.

The study had a cross-over design with two (2) study groups: A and B. Both groups used the study Device (ProVate Device) and the Control device (a commercially available vaginal ring pessary) in a cross-over fashion. The order of the use of the devices was determined based on initial randomization using a cross-over design. Each portion of the sequence lasted 30 days (±3 days) for post-menopausal subjects, or the length of subject’s menstrual cycle (±3 days, within the range of 26-40 days) for menstruating subjects.

Study population included subjects who had previous experience with any vaginal ring pessary due to POP (market available ring pessaries or ProVate Device), women who never used a ring pessary were excluded due to the length of time required to accommodate with a new vaginal device and the relatively short duration of the device usage phases.

The ProVate Device was provided in six (6) different sizes to accommodate vaginal anatomy. The Control device was provided in the size already used by the subjects or in size range that correlates to the ProVate Device sizes. Each subject used the size suitable for her. If needed, as determined by the investigator, a smaller or larger size was provided. The ProVate Device is a single use, disposable device, intended to be used for up to seven (7) days and replaced with a new one, while the commercial vaginal ring pessary is reusable and is intended for long term use, usually for more than a year, with intermittent removal for cleaning. All subjects in the study received a new clean Control device which they used during the Control phase of the study.

Enrollment and inclusion/exclusion criteria were the same for both study groups. The subjects were randomized to either group A or group B. Following randomization, subjects in group A initially used the ProVate Device, while subjects in group B used a market available commercial vaginal ring pessary. The first usage phase started following a fourteen (14) to sixteen (16) days washout period in which subjects were requested to refrain from using any vaginal device, and follow study restrictions (see section 2.1.7). Between the test phases of the two (2) studied devices there was again a washout period of fourteen (14) to sixteen (16) days for post-menopausal subjects or one (1) menstrual cycle for pre-menopausal subjects. Size fitting of each pessary type (ProVate Device and a commercial vaginal ring pessary) was performed at the beginning of each phase, followed by regular use of the chosen size for the rest of the ~30 usage days, or the length of the subject’s regular menstrual cycle.

Vaginal pessary usage length may have differed between subjects, based their preference, lifestyle, specific prolapse, and symptoms’ severity. This is consistent with the device's intended use. Study subjects were encouraged to use the device throughout the whole length of each phase (avoiding use during menstruation and certain illness days). However, for the purpose of this study, each subject was instructed to use the ProVate Device or the commercial vaginal ring pessary for at least 18 days out of the device usage phases of the study (~30 days).

There were seven (7) scheduled visits to the study clinic for each subject; one (1) screening visit and three (3) additional visits for each pessary tested (baseline/size fitting, size confirmation and end of phase visits). Randomization took place during Visit 2 prior to the use of any study device. A schematic representation of the study design is presented in Figure 3.

Unscheduled visits to the clinic took place in cases such as adverse events, need to repeat vaginal pessary size fitting, need to repeat subject’s training on how to insert or remove the ProVate Device etc., as determined by the Principal Investigator.

### Inclusion/Exclusion Criteria

Inclusion/Exclusion evaluation was based on the subject's medical background, pelvic examination, and laboratory tests results conducted at the screening visit. Once eligibility for the study was determined, the candidate was offered to participate in the study and attend visit 2.

**Inclusion Criteria:**

1. Subject is female, aged 21 to 80 years
2. On examination, presence of a vaginal wall prolapse with POP-Q stage 2 – 4 of one (1) or more sites
3. Subject has the ability to understand the nature of the study and consent by signing a written informed consent form
4. Ability to use both hands and insert a Device into the vagina
5. Subject has experience with the use of ring pessary, based on self-report
6. One of the available sizes of the ProVatevaginal Device or commercial vaginal ring pessary is well fitted and can be retained (Visit 2)
7. Normal Pap smear (cervical cytology), within past 24 months (prior to and/or during screening). A Pap smear is not required for women who have had a hysterectomy
8. History of regular menstrual cycles during the previous 6 months lasting approximately 26-40 days, or complete absence of periods (menopause, minimum 6 months since last menstrual period, or post-surgical or premenopausal subject treated with Progesterone IUD.
9. Willingness to use only the study supplied ProVate vaginal Device/Control device
10. Willingness to use only the study supplied lubricant gel, tampons, menstrual pads and daily pads (Panty liner) as applicable
11. Subject agrees not to use douching substances, vaginal medications (other than hormonal local cream – such as estrogen – used prior to the study), suppositories, or vaginal devices including tampons and pads (other than those supplied by the site) during her participation in the study
12. Subject agrees not to apply feminine deodorant spray, powders, perfumes, moistened wipes, lotions, creams or emollients to the genital area for at least forty-eight (48) hours prior to any scheduled study visit. Subject confirms that she did not use any of these products for forty-eight (48) hours prior to screening and randomization
13. Subject agrees not to use any new products (other than those provided by the site) in the vaginal/perineal area at least one (1) week prior to the study start and during her participation in the study
14. Subject agrees not to have sexual activity for at least forty-eight (48) hours prior to any scheduled study visit and confirms that she did not have any sexual activity in the forty-eight (48) hours prior to the study start (screening visit) and randomization visit
15. Subject also agrees not to receive oral and or anal sex or use sexual implements during her participation in the study
16. Subject agrees to continue using the same contraception method she has been using for at least the past three (3) months, for the duration of the study, with the exception of spermicides and condoms or sponges coated with spermicides which should be refrained from, and should not start using any new contraceptive method other than condoms without spermicides supplied by the site
17. Subject who is using local or systemic hormone therapy such as systemic HRT or estrogen cream agrees to continue using the same product she has been using for at least the past one (1) month prior to the study, for the duration of the study. Subject who is not using hormone therapy agrees not to start using such treatment during her participation in the study
18. Subject agrees to undergo all study procedures, including vaginal exams and agrees to follow all study instructions and return for scheduled appointments

**Exclusion Criteria**

1. Subject is pregnant, suspected to be pregnant or planning to become pregnant during the course of the study
2. Subject with present confirmed or suspected vaginal laceration, abrasions and/or ulcerations, as determined by the investigator (or designees) at the screening visit
3. Subject has a symptomatic urinary tract infection as determined by self report and/or lab results
4. Subject has a history of any of the following diagnoses based on self-report: insulin dependent diabetes, Genital Herpes (HSV), Human Immunodeficiency Virus (HIV), Pelvic Inflammatory Disease (PID), Toxic Shock Syndrome (TSS)
5. Subject has a medical condition which might compromise her immune system functions (such as currently treated cancer, immunosuppressive therapy, leucopenia, HIV-positive, or organ transplant)
6. Subject has used corticosteroid medications (systemic or topical) for fourteen (14) or more consecutive days within the last sixty (60) days. Use of intranasal steroids for seasonal allergies or steroidal creams for local dermatitis is allowed.
7. Subject has used antibiotics and/or antifungal and/or antiparasitic medication within two (2) weeks prior to Visit 1 or Visit 2
8. Subject has used spermicides, including condoms coated with spermicides, within seven (7) days prior to Visit 1 or Visit 2
9. Subject has been clinically determined to have or is suspected of currently having or within the last four (4) weeks genital infection (such as bacterial vaginosis, *Trichomoniasis*, yeast infection, *Chlamydia trachomatis*, *Neisseria gonorrhea*) at Visit 1 (screening) or Visit 2 (detected prior to randomization)
10. Subject with repeated vaginal infections (3 or more vaginal infections within last 12 months)
11. Subject has a history of recurrent urinary tract infections (3 or more urinary tract infections within last 12 months)
12. Subject has any co-morbid conditions or any other medical condition or history, as determined by the investigator that could limit the subject’s ability to participate in the study, impact the scientific integrity of the study or may compromise the study results or subject’s safety
13. Subject has severe atrophic vagina as determined by clinical assessment
14. Subject has past difficulty with use of vaginal tampons (insertion, removal and ability to retain a tampon) or vaginal pessaries
15. Subject is currently participating in another clinical study that may directly or indirectly affect the results of this study
16. Subject has given vaginal birth in the last 3 months
17. Subject had abnormal vaginal bleeding during past 6 month
18. Subject recently had vaginal surgery (in the last 3 months)

Figure 3 - A graphic display of the study plan

**Overall Cross over Study Design**

**Screening**

* ICF
* Inc./Exc.
* Vaginal exam
* Lab tests
* Training (restrictions)

**Screening & Enrollment (Visit 2)**

* Retain
* Randomization

**Baseline (Visit 2/5)**

* Sizing &
Vaginal exam
* Baseline swab
* Training

**Weekly follow-up phone call**

To verify:

* Compliance with protocol and study restrictions
* AE’s

**End study**

* End swab
* Vaginal exam

Day 0

At least 18 usage days out of 30 study days/ one (1) menstrual cycle

Day 14/Day 58

Washout 14-16 days

Day 17/61

**Confirmation**

* Vaginal examination to exclude vaginal wall trauma, etc.

Day 44/88

Day 98

Pre- screen phone call

Visit 1

Visit 2/5

Visit 3/6

Visit 4/7

Follow up phone call

**Washout** 14-16 days / 1 menstrual cycle - between visit 4 and visit 5

10- 14 days

Washout

**14-16 days / 1 menstrual cycle**

Pessary

*ProVate*

Washout

 14-16 days

**Portion 1: 30 days/ 1 menstrual cycle**

Follow up phone call

**Portion 2: 30 days/ 1 menstrual cycle**

Pessary

*ProVate*

### General Study Procedures

* Written Informed Consent was obtained from each subject participating in this study (Visit 1), prior to enrollment or initiation of any testing, examination, or review of medical history files for the purpose of screening for this study
* All data collected during the study was recorded in the Prescreening Log, Screening and Enrollment Log, study forms, subjects file, clinic forms and in the CRF
* No tests or study activities were performed during subjects’ menstrual period or during any illness that might have interfered with the study procedures (such as diarrhea). In cases of AEs, illness days, unexpected menstruation or a long vacation which required a temporary halt of usage, the investigator, following consultation with the CRA and Sponsor, was able to decide to prolong or delay the usage period for a specific subject for more than 30 days or more than the lengths of one (1) menstrual cycle
* If the screening (Visit 1) and randomization visits )Visit 2) were more than 16 days apart, the subject was scheduled for another visit to repeat the vaginal culture, urine test and pregnancy test (when applicable) performed at screening. If it was known in advance, during Visit 1, that the subject cannot return to the clinic within 16 days following screening visit for randomization, then vaginal culture, urine test and pregnancy test (when applicable) were not performed at screening. A designated visit for collecting the laboratory samples/pregnancy test was scheduled
* Study subjects were encouraged to use the device throughout the entire length of each phase (avoiding menstruation and certain illness days). However for the purpose of this study, each subject was instructed to use the ProVate Device or the commercial vaginal ring pessary for at least 18 days out of the device usage portion of the study (~30 days). The subject used the Device in her everyday environment, i.e. insertion and removal at home, not at the research center, except when requiring physician’s assistance in removal and re-insertion of the Control device for cleaning or insertion and removal of the study device
* Weekly (or more frequent, based on subject’s compliance) phone interviews by the study staff was conducted in order to record any potential adverse events, to determine if there were any changes in the subject’s health or medications and to verify compliance with study procedures including device usage frequency and study restrictions
* Unless specifically defined, study activities during the usage phases, including device usage, may have been performed on consecutive or non-consecutive days. However, each device usage length (the time elapsed between insertion and removal), should have been at least 24 hours
* In the case where a sign of clinically meaningful vaginal wall trauma was observed by the investigator, the use of additional device was halted for a period determined by the investigator. This was applicable to both study phases
* Four (4) key microorganisms were evaluated during the study:
	+ *Gardnerella vaginalis*
	+ Lactobacillus spp.
	+ *Staphylococcus aureus*
	+ Candida morphotypes

Samples marked with the subject’s initials and identification number were collected and sent to the microbiology laboratory for evaluation and/or quantification.

Lactobacillus spp *and Gardnerella vaginalis* were evaluated by Gram stain using Nugent Semi-quantitative scale based on "Isenberg Handbook of Clinical > Microbiology (ASM), third edition, Appendix 3.2.1-3”.

Candidamorphotypes were evaluated by Gram stain using Semi-quantitative scale.

*Staphylococcus aureus* was evaluated using a Semi-quantitative blood agar culture method.

### Study Instructions

**General Instructions**

During device usage phases, the subject was instructed:

* To use each single ProVate vaginal Device for at least 24 hours and up to seven (7) days
* Not to use the ProVate vaginal Device during her period (menstruation) days
* Not to use the ProVate Device or the Control device during an illness that might have interfered with the study procedures (such as diarrhea)
* To adhere to the directions provided in the Instruction For Use (IFU) leaflet of the device used
* In cases of discomfort during insertion of the vaginal pessary (ProVate Device or Control device), the subject was able to apply lubricant gel to the device prior to insertion. Use of lubricant gel was recorded in the subject diary and CRF
* To complete a usage diary including dates of Device insertion and removal, device details, usage of lubricant, use of contraceptives, menses days etc.
* To report to the study team (during the follow up phone call) of cases where the subject started a new treatment or a new drug or breached the restrictions.

**Study Restrictions**

During all study phases (e.g. screening, washout, randomization (enrollment), size confirmation, and Control/study device usage phases) all study subjects was instructed to strictly adhere to the following instructions and report the study site immediately in case of any deviation:

* Use only pads, tampons, condoms and the lubricant gel provided by the study site
* Use study products (ProVate vaginal Device or Control vaginal ring pessary) as directed
* Not to use vaginal douching substances, vaginal medications (other than local vaginal hormonal therapy, such as estrogen cream used prior to the study and study products provided by the site), suppositories, or vaginal devices
* Not to use new contraceptives other than condoms without spermicides supplied by the site
* Not to apply feminine deodorant spray, powders, perfumes, wipes, lotions, creams or emollients to the genital area for at least 48 hours prior to any scheduled study visit, including screening visit
* Not to try any new products (other than those provided to her by the site) in the vaginal/perineal area at least ten (10) days prior to the study start (Visit 1) and for the whole duration of the study
* Not to use any new estrogen vaginal cream in the vaginal/perineal area or systemic HRT, for at least thirty (30) days prior to the study start (Visit 1) and for the whole duration of the study
* Not to have sexual activity for at least forty-eight (48) hours prior to any scheduled study visit, including screening visit and not to receive oral and/or anal sex for the duration of the study. Subject will also be instructed to refrain from the use of sexual implements for the duration of the study

### Detailed Study Sequence

There were seven (7) visits to the study site for each subject: screening, randomization and start of phase 1, size confirmation and end of phase 1. Following a washout period, start of phase 2 began with visit 5, followed by size confirmation visit, and then end of phase 2 visit.

Initial eligibility screening (day -30 to 0) by phone interview was performed among potential subjects to evaluate their suitability for the study, before summoning them to the clinic. Once potential eligibility for the study was determined based on the study's inclusion/exclusion criteria, the candidate was invited to the study clinic. The subject was instructed not to use vaginal cosmetics for at least forty-eight (48) hours prior to her scheduled visit and meet all the restrictions that are listed in the study restrictions which must be maintained prior to Visit 1.

Screening was held at Visit 1 and the initial part of Visit 2. Subject randomization was performed at the beginning of Visit 2, during screening, prior to the use of any study or Control device.

1. **Visit 1 (day 1) –**

Subjects who were determined to be eligible to start the screening phase were invited to the clinic to receive a comprehensive explanation on the ProVate vaginal Device and the study procedures, and were asked to undergo the screening assessments.

All potential study subjects were evaluated as follows:

* Written Informed Consent was obtained from each candidate prior to initiating testing, examination or review and recording of medical history
* Inclusion/Exclusion Criteria were assessed by the investigator based on the subject's medical history, pelvic and vaginal examination, and laboratory tests results:
	+ Menstrual and menopause status – Menstrual and menopausal status assessment were done by preliminary inquiry by the investigator. Menstrual cycles must be regular (see inclusion criteria). Menopause will be regarded as the absence of menses for at least 6 months.
	+ Pregnancy status – Women of childbearing potential must have a negative pregnancy (urinary b-hCG) test, performed at the clinic, in order to be eligible for the study. In case of pregnancy occurring during participation in the study, the subject must notify the site immediately and she will be withdrawn from the study. Adverse events that occur during subjects’ pregnancy will be recorded. Pregnancy test will be repeated at visit 5.
	+ A vaginal examination will be performed to assess signs of vaginal wall prolapse, vaginal atrophy, vaginal infection, vaginal tenderness and signs of vaginal wall trauma.
	+ POP – assessment of the stage of vaginal wall prolapse
	+ General urine, urine culture, and vaginal screening microbial testing were conducted in order to rule out urinary and/or urogenital infection.
	+ Pap smear results (normal cervical cytology within the 24 months prior to start of the study).
	+ Subjects should continue using the same contraception (e.g., intrauterine device, oral, or injectable contraceptives, etc.) she was using for the past three (3) months, excluding spermicides. Subjects should not use any new contraceptive with the exception of changing from condoms with spermicides to condoms without spermicides supplied by the site.
	+ Subject should continue using the same hormone therapy (local or systemic) she has been using for at least the past one (1) month prior to the study.

After Visit 1 laboratory results were obtained, the investigator evaluated lab results and determined whether the subject was eligible to participate in the study based on the inclusion/exclusion criteria evaluated so far. Eligible subjects were invited to the clinic for the end of screening/ enrollment visit (Visit 2).

If the screening (Visit 1) and randomization visits were more than 16 days apart, the subject had to be scheduled for an additional visit to repeat vaginal culture, urine test and pregnancy test (when applicable) performed at Screening. If it was known in advance, during Visit 1, that the subject would not be able to return to the clinic within 16 days following screening visit for randomization, then vaginal culture, urine test and pregnancy test (when applicable) were not performed at screening. A designated visit for collecting the laboratory samples/pregnancy test was scheduled.

In premenopausal women, Visit 2 was scheduled at least five (5) days prior to the subject’s expected monthly period in order to allow for size confirmation visit (Visit 3 and Visit 6) 3-6 days following size evaluation and allow for a continuous pessary use between these visits.

By the end of visit 1, the subject was reminded again of the study restrictions, and was invited for visit 2. A follow up phone call(s) were held with the subject between Visit 1 and Visit 2 to verify compliance with study restrictions.

1. **Visit 2 (days 14-16)**

Randomization: upon arrival at the clinic, subject was randomized, by means of a statistician-generated randomization list, using a 1:1 ratio into either group A or group B. Group A started the study while using the ProVate Device, while group B started using Control Device.

End of screening: following randomization, the investigator assessed inclusion criterion #6 by evaluation of the required pessary size, based on subject’s vaginal anatomy and, when applicable, the size of the ring pessary previously used by the subject. The investigator then inserted the required device (ProVate Device or the Control device based on the randomization)of the evaluated size into the subject’s vagina to assess ability of the subject to retain the pessary.

Based on visit 1 laboratory results, analysis of subject's medical history, vaginal examination and ability to retain a pessary, the investigator verified the eligibility of the subject to the study. Once eligibility, including the subject’s willingness to continue, has been determined, the subject enrolled into the study.

Enrollment: an initial vaginal microflora testing by Gram stain and *Staphylococcus aureus* Semi-quantitative blood agar culture method was taken prior to pessary size evaluation (in order not to contaminate the swabs). This test sample was sent to the laboratory only if the subject is found to be eligible for the study.

Enrollment was recorded within the screening and enrollment log and CRF. All study subjects completed the following:

* Baseline vaginal examination including evaluation of signs and symptoms which may indicate vaginal infection, ulceration or laceration
* Vaginal microflora baseline test was performed for evaluating the counts of the four (4) key vaginal microorganisms described above.
* In premenopausal women, the study was initiated at least five (5) days prior to the subject’s expected monthly period.
* Subjects clinically determined to have or suspected of having vaginal infection were withdrawn from the study.

1. **Visit 2 & Visit 5:**
* Except for randomization and end of screening, visits 2 and 5 are basically the same and constitute the beginning of each study phase, either using the ProVate Device first and then the commercial pessary – or vice versa
* Subjects received study instructions including study restrictions, how to complete the usage diaries, who should she contact in case of a problem, and information on study visits, device usage, return of used and unused ProVate Devices, etc.
* Vaginal examination was carried our prior to using a specific device:
* Evaluation of signs and symptoms that may indicate vaginal infection
* Evaluation of POP grade and vaginal wall trauma
* Baseline microbial testing (vaginal microflora swab samples) and size fitting
* Subject training
* Pregnancy test (urinary b-hCG) was also conducted for women of childbearing potential in visit 5

Subject training:

Subject received study instructions including study restrictions, how to complete the usage diaries, how to send a copy of the usage diary to the clinic, follow up phone calls by the clinic, who should she contact in case of a problem, and information on study visits, device usage, return of used and unused ProVate Devices, etc.

At the end of the visit the subject received:

* ProVate Devices for home use of the size initially determined for her by the investigator and additional 2 sizes, one (1) smaller and one (1) larger (for subjects in group A).
* Lubricant gel (to be used at subject’s discretion)
* Usage Diary
* Study supplies such as pads, tampons, etc. if needed
1. **Visit 3 & 6 – Size Confirmation (Visit 3 – Day [17-22] and Visit 6 – Day [61-66])**

Following 3-6 study days of home use of the ProVate Device or the Control device, the subject returned to the clinic, with the device in-situ, for an additional vaginal examination (Visit 3 (phase 1) or Visit 6 (phase 2)), in order to evaluate and confirm the suitability of the size fitted.

The investigator performed a vaginal examination and excluded any signs of vaginal wall trauma or infection. The subject (in subjects using the ProVate Device) or the investigator (in subjects using theControl device and cannot remove the ring pessary by themselves) removed the device and the investigator performed an additional vaginal examination without the device. If following the vaginal examination the size was determined to be appropriate for the subject, the subject received a supply of ProVate Devices of the chosen size, or continued using the same Control device.

If the investigator determined that a new size should be fitted, the subject repeated the short term usage and size confirmation stages until a suitable size, out of the available sizes, was found. In case none of the available sizes could be fitted, the subject was withdrawn from the study.

During all study phases, the investigator evaluated the need for a new size if one or more of the following occurred:

* Substantial discomfort
* Abnormal bleeding or pain
* The Device did not remain in the vagina for the required length of use due to repeated expulsion or spontaneous collapse
* By the end of use the Device did not collapse upon a pull of the removal string (ProVate Device only). However, in some cases, due to vaginal anatomy, a well fitted ProVate Device may not collapse upon a pull of the removal string, without causing any discomfort. In such cases, the Device size should not be changed.

All unused and used ProVate Devices were returned to the study site.

During the usage period, the subject was able to come to the study site every two (2) days for removal, cleaning and insertion of the Control device and removal and insertion of the ProVate Device, if desired.

1. **Visits 4 & 7 - End of phase/study (Visits 4 – Day [44-46] and 7 – Day [88-90]**

At the end of the usage period of each phase (portion of the study), following approximately 30 study days, (30±**3** days or one (1) menstrual cycle) the subject returned to the clinic for a scheduled visit..

All subjects underwent vaginal examination and microbial testing (vaginal microflora swab samples, similar to the samples taken at visit 2).

During the visit, the subject returned filled-in usage diaries, and unused and used ProVate Devices. The usage diary was reviewed for accuracy and completeness together with the subject, during the visit.

1. **Post Study Evaluation (Day 98-104)**

Follow up phone call by the study team took place 10-14 days following the end of the second usage phase (study completion, visit 7).

### Description of Specific Procedures within the Study

**1. Interpretation of microbiological results – screening visit**

Purpose: To provide guidelines for consistent evaluation of Visit 1 laboratory test results, in the context of the vaginal examination (signs) and subjects’ self-report of symptoms, in order to determine subjects’ eligibility for the study, for the evaluation of exclusion criteria 3 & 9.

The following microorganisms were evaluated by the screening laboratory (Quest / AML, US /Israeli site, respectively):

**a. By plating (cultures) and microscope (morphotypes):**

*1. Candida [Morphotypes (Quest)/ Albicans & Glabrata (AML)]*

*2. Gardnerella Vaginalis*

*3. Staphylococcus. aureus*

Results of the above microorganisms (1-3) were reported using a semi quantitative scale (None, Few, Moderate, Many, or equivalent scale)

**b. Using nucleic acid based kits:**

*4. Trichomonas vaginalis*

*5. Chlamydia trachomatis*

*6. Neisseria gonorrhea*

Results of the above microorganisms (4-6) were qualitative, reported as Negative/Positive (or equivalent terms).

Vaginal microflora results evaluation:

I. If *Neisseria* *gonorrhea* and/or *Trichomonas vaginalis*, and/or *Chlamydia* *trachomatis* were present (any amount), the subject was excluded from the study regardless of the vaginal examination results.

II. The results of Candida*, Gardnerella Vaginalis* and *Staphylococcus aureus* were interpreted in the context of the physical examination findings (such as heavy discharge, irritation etc.) and urogenital infection symptoms reported by the subject (such as itching, etc.):

a. The mere presence of Candida*, Gardnerella Vaginalis* and *Staphylococcus aureus*, in any amount (Few, Moderate or Many) was not considered as vaginal infection in the absence of urogenital signs or symptoms.

b. If signs and symptoms of urogenital infection were present while none of the above microorganism was detected by the laboratory, a clinical microbiologist was involved.

Table 1 below summarizes the above information.

Table 1-Interpretation of clinical data and lab results at screening

|  |  |  |  |
| --- | --- | --- | --- |
| **Presence of microorganism** | **Signs of vaginal infection** | **Symptoms of vaginal infection (self-report)** | **Conclusion** |
| Yes | Yes | Yes | **Exclude subject.** |
| Yes | No | No | Subject is eligible for the study in the context of Exclusion Criteria #9 |
| No | Yes | Yes | Consult with clinical microbiologist |
| No | Yes | No | Consult with clinical microbiologist |

**2. The Nugent Score - calculation**

Counts of Lactobacillus and Gardnerella were assessed using the Nugent Score. The Nugent Score is a [Gram stain](http://en.wikipedia.org/wiki/Gram_stain) scoring system developed to diagnose bacterial [vaginosis](http://en.wikipedia.org/wiki/Vaginosis) (Nugent, Krohn and Hillier[[82]](#endnote-82)). The score is calculated by assessing the presence of large [Gram-positive](http://en.wikipedia.org/wiki/Gram-positive) [rods](http://en.wikipedia.org/wiki/Rod-shaped_bacteria) ([Lactobacillus](http://en.wikipedia.org/wiki/Lactobacillus) Morphotypes, scored as 0 to 4), small Gram-variable rods ([*Gardnerella vaginalis*](http://en.wikipedia.org/wiki/Gardnerella_vaginalis) morphotypes; scored as 0 to 4), and curved Gram-variable rods (Prevotella, [Mobiluncus](http://en.wikipedia.org/wiki/Mobiluncus) spp. morphotypes; scored as 0 to 2) and can range from 0 to 10.

Table 2-Microbial counts evaluation (Lactobacillus and Gardnerella)

|  |  |  |  |
| --- | --- | --- | --- |
| **Microorganism** | **Method of Evaluation** | **Scale on which Measured** | **Comment** |
| Lactobacillus spp | Gram Stain(Units per HPF) | **0-4**0= Many (>30 CFU/HPF)1= Moderate (6-30 CFU/HPE)2= Few (1-5 CFU/HPE)3= Rare (<1 CFU/HPE)4= None | 1 CFU/HPF correlates to 10e4 CFU/gram. |
| *Gardnerella vaginalis* | Gram Stain(Units per HPF) | **0-4**0= None,1= Rare (<1 CFU/HPE),2= Few (1-5 CFU/HPE),3= Moderate (6-30 CFU/HPE)4= Many (>30 CFU/HPF) | 1 CFU/HPF = 10e4CFU/gram. |

Table 2 above enumerates the scale in which Lactobacillus and Gardnerella were scored.

The following Table 3, taken from Eisenberg’s Handbook of Clinical Microbiology21, provides a display of the Nugent score calculation:

Standardized scoring method for evaluation of Gram stains for BV

Table 3-The Nugent Score

|  |  |
| --- | --- |
| **Quantitation of bacterial morphotype"** | **Points scored per morphotype** |
| **None** | **1+** | **2+** | **3+** | **4+** |
| Medium to large gram-positive rods | 4 | 3 | 2 | 1 | 0 |
| Small gram-negative or –variable rods | 0 | 1 | 2 | 3 | 4 |
| Curved gram-negative or –variable rods | 0 | 1 | 1 | 2 | 2 |

Modified from Nugent et al. using quantitation from the Gram stain procedure.

Interpret as follows: 0 to 3, normal; 4 to 6, intermediate; and 7 to 10, BV.

**3. Candida *spp* counts evaluation**

Table 4 below enumerates the microbial counts evaluation for Candida *spp*

Table 4-Microbial counts evaluation (Candida)

|  |  |  |  |
| --- | --- | --- | --- |
| **Microorganism** | **Method of Evaluation** | **Scale on which Measured** | **Comment** |
| Candida spp | Gram Stain(Units per HPF) | **0-4**0= None,1= Rare (<1 CFU/HPE),2= Few (1-5 CFU/HPE),3= Moderate (6-30 CFU/HPE)4= Many (>30 CFU/HPF) | 1 CFU/HPF correlates to 10e4 CFU/gram. |

**4.Staph aureus counts evaluation**

Table 5 below enumerates microbial counts evaluation for *Staphylococcus aureus*

Table 5-Microbial counts evaluation (Staphylococcus aureus)

|  |  |  |  |
| --- | --- | --- | --- |
| **Microorganism** | **Method of Evaluation** | **Scale on which Measured** | **Comment** |
| *Staphylococcus aureus* | Semi QuantitativePlate Count | **0-4**0= None, 1= Rare (<1 CFU/Plate),2= Few (1-5 CFU/ Plate ), 3= Moderate (6-30 CFU/ Plate) 4= Many (>30 CFU/ Plate) |  |

**2. Interpretation of microbiological results – other study visits**

Vaginal Microflora test samples were taken for all eligible subjects during four (4) different study visits:

* Visit 2 baseline of first usage period of study (either ProVate or Control device, based on the study group A or B). The sample was taken prior to the use of the tested device.
* Visit 4, The end of the first usage period;
* Visit 5, baseline of the second usage period of the study (either ProVate or Control Device). The sample was taken prior to the use of the second tested device.
* Visit 7, following second round of study (either ProVate or Control Device).

The PT-104 Study was designed as a cross over study in which a comparison was made between microflora results, per subject, from before using a specific device (baseline) and the visit following device usage.

Microflora safety analyses were conducted using the per-protocol (PP) population. Results related to Lactobacillus, *Gardnerella Vaginalis* and Yeasts Morphotypes were based on Gram stain and presented using a quantitative Scale (0 to 4+). Nugent score criteria (evaluation of Lactobacillus, and *Gardnerella Vaginalis*) are based on the Isenberg Handbook of Clinical Microbiology ((American Society for Microbiology - ASM), third edition, Appendix 3.2.1-3. Results of *Staphylococcus aureus* are based on Semi Quantitative Plate Count.

In one (1) case (subject 04-021 visit 5 of the ProVate phase) the tube containing the vaginal sample for *staphylococcus* *aureus* was broken during shipment to the laboratory and *Staphylococcus aureus* was evaluated by Covance based on absence of Gram positive cocci in the Gram stain slide.

Two (2) microorganisms – Lactobacillus and *Gardnerella Vaginalis* – are related as they both relate to bacterial vaginosis, and therefore, it is important to combine the change of these two (2) microorganisms in order to determine if the subject has Bacterial Vaginosis. To describe Lactobacillus and Gardnerella counts simultaneously, Nugent Score (scale 0 to 10) was used.

Gram-stain was evaluated by Covance laboratory according to the routine method of testing and reporting the Nugent score. Nugent Score is calculated using 3 microorganisms: Lactobacillus Morphotype, Gardnerella Morphotype and curved rods. Significant changes were calculated directly based on the Nugent scale without transforming the scale into CFU/gr (log count) units.

In premenopausal women, vaginal microflora might depend on the relative day of the menstrual cycle. To minimize the impact of this on study results, the microbial swabs were taken on the same relative day (± 2 days) of each subject’s menstrual cycle.

The following definitions were used while analyzing the primary endpoint:

* A significant Nugent score is any Nugent score ≥ 7 at the end of usage phase (visit 4 or visit 7, however if a subject had Nugent score ≥ 7 at the start of the usage phase (visit 2 or visit 5) she was not considered a failure when she had score ≥ 7 at the end of the phase.
* For *Staphylococcus aureus* and Candida, change was considered significant & meaningful if quantity scale has changed by more than one (1) unit (*i.e*., at least 2 units, for example from 0 to 2+ or from 2+ to 0).
* Safety population was defined as all randomized subjects who used at least one (1) device (ProVate Device or control device) for any duration of time.
* Study Completers are defined as those patients who completed the 7 study visits.
* Per Protocol (PP) population were defined as all patients in the Safety Population without any major protocol deviation, who have used each study device for at least 16 days, without any clinical evidence of vaginal infection at enrollment, and have the following microbial testing at Visit 2 prior to starting any usage phase (using either ProVate Device or a Control commercial vaginal ring pessary):
	+ Nugent score <7; or Nugent score ≥ 7 and no signs or symptoms that may indicate vaginal infection, as determined by the investigator;

**Or,**

* + Candida morphotype ≤2 scale units; Or Candida morphotype >2 scale units and no signs or symptoms that may indicate vaginal infection, as determined by the investigator.

### Materials Used Within the Study (Study Supplies)

Subjects were instructed to use only pads, tampons, condoms and Lubricating Gel supplied by the study clinic:

1. The investigational Device:
	1. The ProVate Device Model D (made by ConTIPI Medical ltd) was supplied in its six (6) different sizes.
	2. Market available Milex Ring Pessaries with support, of sizes 57-89 mm (sizes 2-7), corresponding to the sizes of the ProVate Device. A new Control device was dispensed to all subjects, and they were only allowed to use this device.
2. Lubricating Gel: Personal Water Based Lubricant Jelly, made by Johnson & Johnson Inc.
3. Condoms: Durex Avanti Bare Real Feel Lubricated Non Latex Condoms
4. Daily use pads according to the patient needs, may be used together with the ProVate Device /Control Device, and alone during the washout phase:
* Always Xtra Protection Daily Liners, Extra Long
* Always Xtra Protection Daily Liners, Regular
1. Poise Ultra-Thin Incontinence Pads for Women, Light Absorbency,
2. Regular Menstrual products (for menstruating subjects only) manufactures by Procter & Gamble Inc:

Pads

* Always Ultra-Thin Size 1 Regular Pads Without Wings, Unscented
* Always Ultra-Thin Size 1 Pads with Wings, Regular, Unscented
* Always Ultra-Thin Size 2 Super Pads Without Wings, Unscented
* Always Ultra-Thin Size 2 Pads with Wings, Long, Super
* Always Ultra-Thin Fresh Size 4 Overnight Pads With Wings, Scented

Tampons

* Tampax Tampons with Cardboard Applicator, Regular, Unscented
* Tampax Tampons with Cardboard Applicator, Super

## Statistical Analysis

### Sample Size Calculations

Sample size calculations for the current study were based on the primary endpoint, namely the proportion of patients that are considered a failure according to the primary endpoint.

Based on a previously conducted study with the TIPI vaginal Device for stress urinary incontinence (TIPI 007) we initially expected that 14.6% of patients would have a significant change in *Gardnerella vaginalis* and Lactobacillus *spp* following the use of ProVate. Furthermore, based on the same study, we expected that approximately 2.4% of patients would have a significant increase in Candida *spp* following the use of ProVate whereas this proportion would be 3% for *Staphylococcus aureus* based on the PT 103 clinical safety and efficacy study to evaluate the ProVate Device. In order to be conservative, we assumed that the subjects with a significant change in any of the microorganisms of interest would be mutually exclusive (note discussion of microflora fluctuations above in Section 1.3.9), therefore 20% of the total sample would have a significant change in the microflora.

Based on the literature, we also expected that approximately 5% (2% to 8%) of subjects would have an infection with the microorganisms of interest and would, therefore, require treatment.

Finally, using data from the PT103 study we expected that up to 17% of subjects would experience vaginal symptoms related to infection that are bothersome to them.

Assuming a failure rate in the control group of 30%, based on the information above, and an actual difference in failure rate between groups of zero, a sample size of 54 subjects would achieve 80% power at a significance level of 0.025 using a one-sided non-inferiority test of correlated proportions (McNemar test assuming 10% discordant pairs), and a non-inferiority margin of 15%[[83]](#endnote-83),[[84]](#endnote-84).

### Analysis Populations

The Safety Population is defined as all randomized subjects who used at least one (1) device (ProVate Device or Control device) for any duration of time.

In addition, a Per Protocol (PP) population was defined comprising all randomized subjects who:

* Used the ProVate Device and the commercial vaginal ring pessary for at least 16 days out of each device usage portion of the study (~30 days);
* Had no ‘major’ protocol deviations deemed likely to affect outcome, e.g. had available valid assessments of vaginal microflora during both study phases. Protocol deviations were blindly reviewed and classified as minor vs. major prior to database lock.
* Had no evidence of vaginal infection at enrollment per section 2.1.9.

All analyses of endpoints (primary and secondary) were conducted on the PP population. Safety analyses of AEs were conducted on the Safety Population.

### Analysis of Objectives

For the primary objective of the study, the number and proportion of patients treated with each device that meet the failure criteria was described. Non-inferiority of the ProVate Device was to be declared if the upper limit of a 1-sided 97.5% confidence interval constructed on the difference in proportions (ProVate - commercial vaginal ring pessary), taking into consideration the correlation due to the cross-over design, was less than the non-inferiority limit of 15%. In this analysis, patients with a Nugent score ≥ 7 and either signs or symptoms that may indicate infection, at baseline (Visit 2 and/or Visit 5), were to be excluded for that period.

In addition, the proportion of patients treated with each device meeting each individual failure criterion, as well as the proportion of patients with an increase in microbial count greater than 1 unit scale in any study organism was described with summary statistics.

Shift tables presenting changes between the start and end of each study phase for each of the microflora tested were produced using cross tabulation. Furthermore, the percentage of patients with no change vs. beneficial change vs. non-beneficial change in each microorganism’s scale was described.

Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 21.1. The total number of AEs, and the total number and proportion of patients experiencing at least one (1) AE during each phase were summarized by system organ class (SOC) and preferred term (PT). To count the number of patients who experience each AE, patients experiencing the same AE multiple times were only counted once for the corresponding SOC and PT. AEs were also described as described above for serious AEs (SAEs) and according to causal relationship to the treatment (Remotely Related / Possibly Related / Probably Related / Related).

Blind review

All protocol deviations were classified by the sponsor as major or minor in a blinded manner. Deviations related to impact on microflora, such as medications taken by subjects during the study, were evaluated by a clinical microbiologist who was blinded to results. Subjects with a major deviation were excluded from the PP group analysis.

# Results

The study CRO was JSS Medical Research (Montreal, Canada). Microbiological assessments of study endpoints were performed by Covance Labs Inc. (Indianapolis, IN) and screening laboratory tests were performed by Quest Diagnostics Inc. (Secaucus, NJ) for the US sites and by AML Labs (Hertzelia, Israel) for the Israeli site.

## The Subjects – Baseline Characteristics

### Subject Disposition

The PT-104 study was conducted in seven (7 sites – six (6) in the United States of America and one (1) in Israel), between August 2017 and September 2018.

Table 6 shows a list of the 7 study sites, and the number and percentage of subjects in each of the populations (% out of screened population at the site)

Table 6-Number and Percentage of Subjects Included in each Analysis Population

| **Center** | **Screened** | **Randomized** | **Safety****Population** | **Per protocol****Population** |
| --- | --- | --- | --- | --- |
| N | % | N | % | N | % | N | % |
| 1. Morhead city, NC (Dr Vradelis)
 | 15 | 100% | 11 | 73.3% | 11 | 73.3% | 8 | 53.3% |
| 1. Winston-Salem, NC (Dr Parker)
 | 6 | 100% | 4 | 66.6% | 4 | 66.6% | 2 | 33.3% |
| 1. Lake Worth, FL (Dr Lederman)
 | 11 | 100% | 11 | 100% | 11 | 100% | 11 | 100% |
| 1. Hertzelia, Israel (Dr Ziv)
 | 24 | 100% | 22 | 91.6% | 22 | 91.6% | 21 | 87.5% |
| 1. Raleigh, NC (Dr Littleton)
 | 6 | 100% | 4 | 66.6% | 4 | 66.6% | 2 | 33.3% |
| 1. Leesburg, FL (Dr Moffette)
 | 21 | 100% | 19 | 90.4% | 19 | 90.4% | 14 | 66.6% |
| 1. Ft Lauderdale, FL (Dr Zafran)
 | 2 | 100% | 2 | 100% | 2 | 100% | 0 | 0% |
| **All** | **85** | **100.0** | **73** | 85.8% | **73** | 85.8% | **58** | 68.2% |

* 85 subjects were screened for the study. 12 subjects were excluded following visit 1.
* 73 subjects (85.88% of the screened population) were randomized into the study during visit 2.
* 71 subjects were eligible for the study as two (2) randomized subjects (#06-016, 01-011) could not find the correct size of the device for them, hence did not comply with inclusion criteria #6, and were excluded at visit 2.
* Safety population was defined as all randomized subjects who used at least one (1) device (ProVate Device or Control device) for any duration of time. 73 subjects (100% of randomized population) were included in the Safety population;
* Per Protocol (PP) population (58 subjects, 79.5% of randomized population) were defined as all patients in the Safety Population, without any major protocol deviation, who have used each study Device for at least 16 days, without any clinical evidence of vaginal infection at enrollment, and have the following microbial testing at Visit 2 prior to starting any usage phase (using either ProVate Device or a Control commercial vaginal ring pessary):
* Nugent score <7; or Nugent score ≥ 7 and no signs or symptoms that may indicate vaginal infection, as determined by the investigator;

**Or,**

* Candida morphotype ≤2 scale units; Or Candida morphotype >2 scale units and no signs or symptoms that may indicate vaginal infection, as determined by the investigator.
* 59 (80.8%) subjects attended all seven (7) study visits. However, one (1) subject (#02-003) who was included in the Per Protocol (PP) population and attended all seven (7) study visits, terminated usage period of the Control Device earlier than stated in the protocol (see below).
* One (1) subject (#01-003) had one (1) major deviation and was thus excluded from the Per Protocol (PP) population.
* 58 subjects (out of the 59 subjects) who attended all seven (7) study visits were considered completers, including the one (1) subject mentioned above (#02-003) who was included in the Per Protocol (PP) population and attended all seven (7) study visits, but terminated the usage period of the Control Device earlier than stated in the protocol, since she wanted to continue using the pessary she was using prior to the study.

51 subjects (69.8% of the safety population) were recruited in the US sites. Of them, 37 subjects (63.8% of the PP population) were included in the PP population.

22 subjects (30.1% of the safety population) were recruited by the Israeli site. Of them, 21 subjects (36.2% of the PP population) were included in the PP population.

Figure 4below presents a flow chart of subject disposition, including the number of subjects who did not meet all inclusion and exclusion criteria (Screen Failures), and subjects who withdrew or were excluded from the study.

Subjects at Screening N=85

Randomization (Safety group) N=73

Screen Failures (V1) N=12

Enrolled N=71

Screen failures (V2) N=2

Per Protocol Population N=58

Excluded – Major PD N=1

Withdrew N=12

Attended all study visits N=59 \*

Figure 4-Flow Chart of Subject Disposition

\* One subject (02-003) who attended all 7 visits but discontinued early, using Control Device, is included here

Table 7 presents the distribution of reasons for premature termination in the 12 subjects (regardless of the device they used) who were enrolled but were not included in the PP population. Six (6) subjects were withdrawn following visit 2 due to adverse events (2 while using Control Device, 4 while using ProVate Device); three (3) subjects withdrew consent, and three (3) subjects terminated due to protocol violations – one (1) used the wrong brand of Control Device, while two (2) used excluded medications while using the ProVate Device and were withdrawn during the study.

One (1) subject (#01-003) who was considered a study completer was later excluded due to major protocol deviation, by the sponsor blinded review.

The PT-104 study was a cross over study, with two (2) sequence groups – group A in which all subjects used the ProVate Device, and then used the Control Device (A: ProVate Device – Control, 26 subjects in the Per Protocol group), and group B, in which subjects used the Control Device first and then the ProVate Device (B: Control – Device, 32 subjects in the Per Protocol group).

Table 7-Reasons for Premature Termination (Enrolled Subjects)

| **Premature Termination Reason** | **N** | **Percent Out of Prematurely Terminated (N = 12)** | **Percent Out of Randomized (N = 73)** |
| --- | --- | --- | --- |
| Non-serious Adverse Events(ProVate N= 4, Control N=2) | 6 | 50% | 8.2% |
| Protocol violations | 3 | 25% | 4.1% |
| Withdrew Consent | 3 | 25% | 4.1% |
| **All** | **12** | **100.0** | **16.4%** |

In order to explore a potential sequence effect, a logistic regression model was built using the primary endpoint as the dependent variable and device type (ProVate vs. Control) and randomization sequence as independent variables. No significant effect of randomization sequence was observed (p=0.325) suggesting the lack of sequence effect and allowing the pooling of the results for each device from both sequences in all analyses.

### Subjects’ Demographics (PP Subjects)

All eligible randomized participants in the study were female subjects, and they all had Pelvic Organ Prolapse assessed by vaginal examination. At screening, and before introduction of any vaginal device, 21 (36.2%) of the 58 subjects in the PP population had POP-Q stage 2 prolapse, 35 (60.3%) had POP-Q stage 3 prolapse and 2 (3.4%) had POP-Q stage 4 prolapse.

All eligible subjects had experience with using a vaginal ring pessary – whether commercially available ring and/or the ProVate Device. The duration of usage of the pessaries varied between 1 month and 15 years. 17 subjects (29.3%) used a ring pessary on a continuous basis, while 41 (70.7%) had non-continuous use.

The mean age was 64.5±10.57 (range 36-77 years), out of which 2 (3.4%) were 31-40 years, 4 (6.9%) between 41-50 years, 12 (20.7%) between 51-60 years, 19 (32.8%) between 61-70 years and 21 (36.2%) were between 71-80 years old.

47 subjects (81.0%) were non-Hispanic or Latino, 10 (17.2%) were Hispanic or Latino, and in 1 case data was missing (as subject refused to discuss). 53 (91.4%) of subjects were white, 2 (3.4%), were African-American, and 2 (3.4%) were American Indian or Alaska Native. In one (1) case the data was missing as subject refused to discuss (same subject as above).

Mean weight of subjects was 73.9±16.16 Kgs (range 53-139.3 Kg), with mean height 161.7±6.68 cm (range 143-175.3), and mean Body Mass Index (BMI) of 28.2±5.47 (range 19.7-46.7).

All subjects were fully active and fully aware during their daily life, and none had impaired mobility or dementia. 16 (27.6%) were doing regular physical activities, including sports, 36 (62.1%) were doing some activity, and 6 (10.3%) considered themselves to be only minimally active.

52 (89.7%) subjects reported various previously diagnosed medical conditions, which did not require exclusion from the study. 43.1% had neurological conditions, 48.3% had metabolic conditions, 15.5% had pulmonary problems, 43.1% had some cardiovascular conditions, and 37.9% reported allergies.

All 58 subjects (100%) had at least one (1) delivery, and average parity was 2.7±1.11 deliveries per subject [range 1-5]. There were neither deliveries nor pregnancies within 12 months prior to screening.

4 (6.9%) subjects were premenopausal; their regular mean menstrual cycle length was 28 days. 53 (91.4%) subjects were postmenopausal (last menstrual period at least 6 months before screening), with a mean amenorrhea period of 17.9±9.35 years (range 1-37 years). 4 of the postmenopausal subjects were using systemic Hormone Replacement Therapy (HRT), 5 vaginal hormone therapy, while 48 were not using any HRT, including local vaginal Estrogen preparation. There were no perimenopausal subjects, and in one (1) case (1.7%) data was missing.

Among the Per Protocol population subjects, there were altogether 16 surgeries during the 5 years prior to the study, but there were no pelvic operations within the 3 months prior to screening. 2 (3.4%) operations were vaginal, while 14 (24.1%) were non-vaginal.

All subjects were inquired regarding history of vaginal infections in the previous 12 months, and time since such last infection was recorded. Five (5) (8.6%) reported having at least one (1) vaginal infection within the previous 12 months. Of them, 3 subjects (5.2%) reported yeast infection, one (1) subject (1.7%) reported one (1) case of Bacterial Vaginosis, and one (1) subject (1.7%) reported infection of unknown etiology, but there were no ongoing vaginal infections at screening and enrolment.

None of the subjects admitted into the study used any medication that may have had an impact on eligibility within the study, such as use of antibiotics, antifungal agents or antiparasitic agents. Other specific therapies, such as steroids and Hormone Replacement Therapy were allowed under certain conditions.

###  Vaginal Status Assessment prior to Device Use

* Prior to enrollment into the study, all premenopausal subjects had beta HCG urinary test to exclude pregnancy. All tests were negative for eligible subjects.
* Thin Prep® cervical cytology was performed on all non-hysterectomized subjects unless they were able to show normal cervical cytology within the previous 24 months.
* Urine cultures and vaginal microbial testing were also carried out prior to enrolment.

Table 8 below shows assessment of vaginal examination prior to initial study phase visits (visit 2 & 5, before using any device). Any self-reported bothersome symptom of vaginal infection or findings by the investigator of signs of vaginal infection were recorded. A summary of the records appears in Table 8, showing that none of the subjects had any self-reported symptoms or vaginal findings prior to any usage phase.

Table 8-Vaginal symptoms and signs prior to device usage (Visit 2 or 5)

|  |  |
| --- | --- |
| **Self-reported symptoms that are bothersome** | **Signs of vaginal infection** |
| Vulvar itching | 0 (0%) | Vaginal irritation | 0 (0%) |
| Vaginal itching | 0 (0%) | Vulvar irritation | 0 (0%) |
| Foul odor | 0 (0%) | Foul odor | 0 (0%) |
| Abnormal discharge | 0 (0%) | Abnormal discharge | 0 (0%) |
| other | 0 (0%) | other | 0 (0%) |
| Investigator’s conclusion =0 (%) bothersome symptoms | Investigator’s conclusion =0 (%) signs & symptoms |

## Study Device Usage Parameters

### Distribution of Device Sizes

Control Device

One US market available reusable Control device (Ring pessary with support by Milex®, Cooper Surgical Inc., Trumbull, CT) was employed during the study, and its length of usage was according to the standard of care at each site. Regardless of each site’s standard of care, each subject was seen at the clinic at least once, during the size confirmation visit, within days 3-6 from the start of usage phase, and at the end of the usage phase (following 30 days) according to the protocol.

ProVate Device

Subjects were allowed to use as many ProVate Devices as they wished as long as they used each Device for at least 24 hours and up to seven (7) days. They were also encouraged to use the ProVate Device as long as they can, within the limit of the seven (7) usage days. Visits to the study site were identical to those held with the Control Device.

Distribution of the various sizes of the devices (both ProVate and Control device) is demonstrated in Figure 5.

Figure 5-Distribution of most suitable device sizes in the study (ProVate & Control devices, PP, percentage per each size)

As may be noted, the most prevalent size of the Control Device used was 70mm (29.3%) and of the ProVate Device was 73mm (39.7%).

### Study Device Exposure

Device exposure was calculated in terms of the number of hours exposed to each device usage phase, excluding usage periods <30 minutes, and limited only to devices that were used at the home environment (excluding devices that were used for short terms at the clinic for sizing and user training), based on the usage diaries filled by the subjects. These short usages of the device during the size trial fittings are not expected to impact vaginal microflora given their short duration of use.

Figure 6 demonstrates percentage of Devices used per each usage length (e.g. – up to 2 days, between 4-6 days, etc.).

Figure 6-Distribution of usage lengths per Device (PP group, ProVate Device, percentage per each time frame)

As may be seen in Figure 6, most usages (62.9%) of a single ProVate Device were for at least 4 days. A single reusable Control Device remained in the vagina during the whole Control Device phase (in most cases for at least 22 days).

Total usage length for both ProVate Device and Control device was calculated in hours, and then divided by 24 in order to show usage in days as well. Cumulative Mean ProVate Device usage length per subject in the Per-protocol population was 681.6 ±85.92 hours, (~ 28.4 ± 3.58 days), whereas mean cumulative Control device usage time was 717.7 ±64.05 hours, (~ 29.9 ± 2.66 days). Calculated mean total (all subjects) usage days for the ProVate Device in the PP population was 1647 days, and 1734 days for the Control device,

Altogether, 383 ProVate Devices were used in the study (in the home environment), by all subjects in the safety population, an average of 5.7 ± 1.58 [range 1-9] Devices per subject.

350 ProVate Devices were used by the PP population (in the home environment), an average of 6.0 ± 1.08 [range 4-9] Devices per subject.

Cumulative length of device usage, in timeframes of 5 days, is shown in Figure 7 below, for both ProVate and Control devices. At least 16 usage days per device, were required in order to include the subject in the PP population.

Figure 7-Distribution of device usage length by time frames (ProVate and Control devices, PP, percentage of usage length)

As may be noted from Figure 7, the vast majority of any device cumulative usage length within the study was above 25 days for both devices.

## The Study Endpoints

### The Primary Endpoint

Analysis of the primary endpoint was conducted only on the PP population. The primary endpoint is based on the following failure criteria:

1. Failure parameter #1 – vaginal infection per lab results:

A subject is a failure if there is a significant change in vaginal microflora (i.e., significant meaningful change in Lactobacillus spp., *Gardnerella vaginalis*, Candida spp, or *Staphylococcus aureus* levels from baseline), where the significant change is defined, according to the common clinical practice as:

* 1. Nugent score < 7 at baseline (Visit 2/5) for whom the end of treatment Nugent score (Visit 4/7) is ≥ 7, or
	2. > 1 scale unit increase in *Staphylococcus aureus,* or
	3. > 1 scale unit increase in Candida morphotype,

or;

1. Failure parameter #2 - bothersome vaginal symptoms or;
2. Failure parameter #3 - vaginal symptoms that require treatment,

Failure is defined as meeting at least 1 of the 3 defined failure parameters above.

Table 9 below provides the number and proportion of subjects who met at least 1 failure criterion, according to the Primary Endpoint, for each of the tested devices, ProVate and Control, while assessing results from the 3 failure parameters above.

Table 9-Number and proportion of subjects with failure for each device

|  |  |
| --- | --- |
|  | **Failure – usage phase** |
| **ProVate (N=58)** | **Control (N=58)**  | **95% Confidence Interval** | **P-value** |
|  | Subjects | % | Subjects | % |
|  **Failure**  | 9 | 15.5 | 9 | 15.5 | -13% : 13% | >0.999 |

The table clearly shows that the total number of subjects who met at least one (1) failure criterion was comparable between ProVate and Control devices; the rate of failure as defined while using the above mentioned 3 failure parameters was 9 (15.5%) for ProVate and 9 (15.5%) for Control (lower than the 30% expected during the design of the study), with a 1-sided 97.5% upper limit of 13%, which was within the non-inferiority limit of 15%. There were two (2) cases of bothersome vaginal complaints (failure parameter #2) and one (1) case which also required treatment) failure parameter #3) for vaginal infection, within the Control group, and none with the ProVate group.

**Table 9 above shows that the Primary Endpoint was met successfully**.

### Secondary Endpoints

The study secondary endpoints are:

* Proportion of subjects with changes (increase or decrease) in microbial counts following device usage, compared to baseline (i.e. Visit 2 for the usage phase 1 and Visit 5 for the usage phase 2), greater than 1 unit scale (> 1 unit scale) in any of the 4 study microorganisms.
* Proportion of patients who have a Nugent score ≥ 7 for Lactobacillus spp. and *Gardnerella vaginalis* following device usage.
* Proportion of patients who have a >1 scale unit increase in *Staphylococcus aureus* following device usage.
* Proportion of subjects who have a >1 scale unit increase in Candida morphotype following device usage.
* Proportion of subjects who have vaginal symptoms that are bothersome following device usage
* Proportion of subjects who have vaginal symptoms that require treatment following device usage.

Table 10 shows summary results for the secondary endpoints in the PP population

Table 10-Summary results for the secondary endpoints

|  |  |
| --- | --- |
| **Failure criteria** | **Usage Period** |
| **ProVate (N,% )** | **Control (N,% )** |
| Patients with a >1 unit scale change (increase or decrease) from baseline to end of usage phase in any studied microorganism | 26 (44.8%) | 21 (36.2%) |
| Patients with Nugent score ≥ 7 following device usage | 4 (6.9%) | 2 (3.4%) |
| Patients with a >1 scale unit increase following device usage in *Staphylococcus aureus*  | 2 (3.4%) | 3 (5.2%) |
| Patients with a >1 scale unit increase following device usage in Candida morphotype | 3 (5.2%) | 2 (3.4%) |
| Patients with vaginal symptoms that are bothersome | 0 (0.0%) | 2 (3.4%) |
| Patients with vaginal symptoms related to vaginal infection that require treatment  | 0 (0.0%) | 1 (1.7%) |
| **Patients with a conclusion of vaginal infection (i.e. physician reported an overall conclusion of vaginal infection)**  | 0 (0.0%) | 2 (3.4%) |

Results from this table show that the various laboratory findings, and symptoms and signs of vaginal infection, are comparable between existing marketed vaginal Control Device and the new ProVate Device, further elaborating on the primary endpoint. However, they also demonstrate the very high rate of fluctuations of vaginal microflora (36-44%), which are well known from the literature, and are further discussed in sections 1 & 4.

## ~~Adverse Events~~

~~General safety analyses were conducted on the Safety analysis population.~~

~~As noted within Table 11 below, altogether there were 85 adverse Events (AEs) during the study, while using both ProVate Device and the Control Device, but also during the two (2) wash-out periods and the post-study period.~~

* ~~AEs reported during the usage of the ProVate Device: 54 AEs occurred in 26 subjects (36.6% of the safety group). 40 of those were somehow device-related, and 32/40 (80%) were anticipated. There were no serious AEs, and all AEs (100%) resolved with no sequelae.~~
* ~~AEs reported during the usage of the Control Device: 31 AEs occurred in 22 subjects (34.3% of the safety group). 17 of those were somehow device-related, and 9/17 (52.9%) were anticipated. There were no serious AEs, and 26/31 (83.8%) resolved with no sequelae.~~

### ~~Serious AEs~~

~~No serious adverse events were reported during the entire study.~~

### ~~Severity~~

~~Within the whole studied population, there were altogether 85 AEs, of which 58 (68.23%, in 45 women) were mild, 22 (25.8%, in 12 subjects) were moderate and 5 (5.8%, in 1 subject) were considered severe by the investigator.~~

~~Most AEs with reasonable causal relationship to the device (i.e. remotely related, possibly related, probably related or related to any of the devices) were of mild (32 subjects, 56.14%) or moderate (19 subjects, 35.08%) severity.~~

~~There were five (5) cases (8.7%) of severe AEs in a single (1) subject (#06-021) across the entire usage phase while using the ProVate Device. The investigator considered her complaints (vaginal odor, daytime urinary frequency (for which she was treated with Hydrochloride Phenazopyride), pelvic pain, vaginal discomfort, pelvic pressure) as severe, while using the ProVate Device. However, she completely recovered from all reported AEs, and completed the study with no complaints or residual effect.~~

### ~~Device-related AEs~~

~~When a subject initially uses any type of a new intra-vaginal device, it is common that the first few days of use are accompanied by some discomfort and, occasionally, other mild adverse events. This is actually a learning and accommodation period. Complaints, or AE’s, which are anticipated at that time of vaginal pessary accommodation or use, will usually include vaginal wall trauma, discomfort, spotting, pain or some bleeding.~~

~~Table 11 below summarizes some characteristics of Adverse Events from the ProVate and the Control groups, in the Safety Population.~~

~~Table 11-Summary of Adverse Events Categorization (Safety population)~~

|  | **~~ProVate (N=71)~~** | **~~Control (N=64)~~** |
| --- | --- | --- |
| **~~Parameter~~** | **~~Nof Events~~** | **~~Nof Patients~~** | **~~%of Patients~~** | **~~Nof Events~~** | **~~Nof Patients~~** | **~~%of Patients~~** |
| ~~TOTAL~~ | ~~54~~ | ~~26~~ | ~~36.6%~~ | ~~31~~ | ~~22~~ | ~~34.4%~~ |
| ~~Anticipated AE~~ | ~~Yes~~ | ~~34~~ | ~~17~~ | ~~23.9%~~ | ~~10~~ | ~~9~~ | ~~14.1%~~ |
| ~~No~~ | ~~20~~ | ~~14~~ | ~~19.7%~~ | ~~21~~ | ~~16~~ | ~~25.0%~~ |
| ~~Ongoing AE~~ | ~~Yes~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ | ~~5~~ | ~~5~~ | ~~7.8%~~ |
| ~~No~~ | ~~54~~ | ~~26~~ | ~~36.6%~~ | ~~26~~ | ~~19~~ | ~~29.7%~~ |
| ~~Intensity / severity~~ | ~~Mild~~ | ~~33~~ | ~~21~~ | ~~29.6%~~ | ~~25~~ | ~~19~~ | ~~29.7%~~ |
| ~~Moderate~~ | ~~16~~ | ~~7~~ | ~~9.9%~~ | ~~6~~ | ~~5~~ | ~~7.8%~~ |
| ~~Severe~~ | ~~5~~ | ~~1~~ | ~~1.4%~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ |
| ~~Serious AE~~ | ~~No~~ | ~~54~~ | ~~26~~ | ~~36.6%~~ | ~~31~~ | ~~22~~ | ~~34.4%~~ |
| ~~Yes~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ |
| ~~Study device relationship~~ | ~~Not study device related~~ | ~~13~~ | ~~11~~ | ~~15.5%~~ | ~~14~~ | ~~11~~ | ~~17.2%~~ |
| ~~Remotely~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ | ~~2~~ | ~~2~~ | ~~3.1%~~ |
| ~~Possible~~ | ~~15~~ | ~~8~~ | ~~11.3%~~ | ~~6~~ | ~~4~~ | ~~6.3%~~ |
| ~~Probable~~ | ~~20~~ | ~~10~~ | ~~14.1%~~ | ~~8~~ | ~~6~~ | ~~9.4%~~ |
| ~~Related~~ | ~~5~~ | ~~3~~ | ~~4.2%~~ | ~~1~~ | ~~1~~ | ~~1.6%~~ |
| ~~unknown~~ | ~~1~~ | ~~1~~ | ~~1.4%~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ |
| ~~Action taken regard study device~~ | ~~None~~ | ~~42~~ | ~~21~~ | ~~29.6%~~ | ~~31~~ | ~~22~~ | ~~34.4%~~ |
| ~~Study Device Delayed~~ | ~~2~~ | ~~2~~ | ~~2.8%~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ |
| ~~Device Discontinued~~ | ~~10~~ | ~~5~~ | ~~7.0%~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ |
| ~~Treatment required~~ | ~~None~~ | ~~41~~ | ~~19~~ | ~~26.8%~~ | ~~21~~ | ~~14~~ | ~~21.9%~~ |
| ~~Drug~~ | ~~13~~ | ~~11~~ | ~~15.5%~~ | ~~10~~ | ~~10~~ | ~~15.6%~~ |
| ~~Subject outcome~~ | ~~Recovered – No Residual Effects~~ | ~~54~~ | ~~26~~ | ~~36.6%~~ | ~~26~~ | ~~19~~ | ~~29.7%~~ |
| ~~AE still present – No treatment~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ | ~~3~~ | ~~3~~ | ~~4.7%~~ |
| ~~AE still present – Being treated~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ | ~~2~~ | ~~2~~ | ~~3.1%~~ |
| ~~Recovered; residual effects present – No treatment~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ |
| ~~Recovered with residual effects present – treated~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ |
| ~~Subject discontinued study~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ |
| ~~Death~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ | ~~0~~ | ~~0~~ | ~~0.0%~~ |

~~Table 12-Distribution of Adverse Events with reasonable causal relationship between ProVate and Control, further divided into body system, anticipated and non-anticipated (Safety population, 8/6 means 8 AEs in 6 subjects)~~

|  |  |  |  |
| --- | --- | --- | --- |
| **~~Body System~~** | **~~Complaint~~** | **~~ProVate~~** | **~~Control~~** |
| **~~Anticipated~~** | **~~Non-anticipated~~** | **~~Anticipated~~** | **~~Non-anticipated~~** |
| ~~Abdomen &~~ | ~~pain~~ | ~~1~~ | ~~3/2~~ |  | ~~1~~ |
|  | ~~Tenderness~~ |  | ~~1~~ |  |  |
| ~~Back~~ | ~~pains~~ |  | ~~1~~ |  |  |
| ~~Urinary Tract~~ | ~~Urgency~~ |  | ~~1~~ |  |  |
|  | ~~Frequency~~ | ~~1~~ |  |  |  |
|  | ~~De-Novo SUI~~ |  |  | ~~1~~ |  |
|  | ~~UTI~~ |  |  | ~~1~~ | ~~1~~ |
| ~~Pelvic~~ | ~~Discomfort~~ | ~~1~~ | ~~1~~ |  | ~~1~~ |
|  | ~~pain~~ |  | ~~1~~ |  |  |
| ~~Vaginal~~ | ~~Discharge~~ | ~~3/2~~ |  | ~~3/3~~ | ~~2/2~~ |
|  | ~~Wall trauma~~ | ~~5/4~~ |  | ~~2/2~~ |  |
|  | ~~Spotting~~ | ~~8/6~~ |  | ~~1~~ |  |
| ~~Odor~~ | ~~2/2~~ |  |  |  |
| ~~Infection~~ |  |  | ~~1~~ |  |
| ~~Granulation tissue~~ | ~~1~~ |  |  |  |
| ~~Discomfort~~ | ~~8/6~~ |  |  |  |
| ~~Vulvovaginal pain~~ | ~~1~~ |  |  |  |
| ~~Swelling~~ | ~~1~~ |  |  |  |
| ~~itching~~ |  |  |  | ~~2/2~~ |
| ~~Vulvar~~ | ~~itching~~ |  |  |  | ~~1~~ |
| ~~Number of AEs~~ | ~~32/16~~ | ~~8/4~~ | ~~9/7~~ | ~~8/6~~ |
|  |  | ~~40/17~~ | ~~17/13~~ |

~~Table 12 shows AEs with reasonable causal relationship to one of the devices (remotely, possibly, probably, related), within the safety population. Altogether there were 57 such AEs in both device populations:~~

* ~~ProVate Device - 40 AEs with reasonable causal relationship to the device in 16 subjects while using 383 fresh single use ProVate Devices. It is not uncommon to note anticipated AEs with reasonable causal relationship to the device such as slight discomfort and spotting, mainly in postmenopausal women using a new vaginal device. These AE’s are considered minor and, based on the medical literature are anticipated when using vaginal devices, such as vaginal pessaries.~~
* ~~Control Device - 17 AEs with reasonable causal relationship to the device in 13 subjects while using the Control device (a single device in each subject).~~

~~All these AEs were comparable, basically minor and mainly anticipated – for both ProVate & Control.~~

~~With ProVate, out of the 40 AEs (safety population, over 383 Devices used) which had reasonable causal relationship to the Device, 34 (85%) were anticipated. As expected, vaginal discomfort & spotting were the most frequent anticipated AEs in this study.~~

~~With Control, out of the 17 AEs (safety population) which had reasonable causal relationship to the device, 10 (58.8%) were anticipated. Vaginal discharge was the most frequent anticipated AE (5 incidents among 5 subjects).~~

**~~Discussion of Anticipated AEs~~**

* ~~Vaginal wall trauma (including erosions, abrasions and lacerations) is the most frequently reported complication of a pessary[[85]](#endnote-85), presenting as foul odor, purulent discharge, irregular blood stained discharge, and increased vaginal fluid. It occurs in ~19.3% of pessary users[[86]](#endnote-86) (range 3-48.2%[[87]](#endnote-87)~~~~,~~~~[[88]](#endnote-88)), and believed to be caused by pressure on vaginal walls or by the initial phase of device usage (accommodation period). Rate of vaginal wall trauma in the PT-104 Microflora study was rather low for both devices, as compared to the literature, with only 4 subjects (5.6%) among the ProVate users (in one (1) subject, the investigator mentioned both erosion and laceration at the same time) and in 2 subjects among the Control users (2.8%). One (1) of the four (4) subjects with vaginal wall trauma (#006-006) discontinued the study while having 2 AEs with ProVate and 3 AEs (including vaginal wall trauma) with Control device. No treatment was needed and she recovered with no residual effect. In subject #006-004, for some reason, the investigator reported both erosion and laceration during the exact same time. She completed the study as planned with no need for treatment, and recovered with no residual effect. The other 2 subjects also completed the study as planned with no treatment and no residual effect. Among subjects who had vaginal wall trauma while using the Control Device, one (1) subject (#006-006) did not require any treatment and recovered completely, while the in the second subject (#006-021) the wall trauma was still ongoing when the study ended. Frequency of vaginal wall trauma for both devices was lower than usually cited in the literature and within the lower range of the regularly cited figures.~~
* ~~Vaginal discomfort & pain – anticipated complaints with any vaginal device, mainly during initial use. With ProVate Device there were 8 complaints on discomfort in 6 subjects (8.4%), and 1 complaint of vulvovaginal pain in one (1) of the 6 subjects who also complained on discomfort. In 2 cases the device was delayed but there was no need for treatment and they concluded the study with no residual effect. One (1) subject (#006-005) terminated the study for 5 different AEs, and there was no way to figure out the exact reason for termination. There were no such complaints with Control Device. Reports of pain in pessary users (and perhaps this includes discomfort within) ranges 6.9-41%~~~~[[89]](#endnote-89),31~~~~, with discomfort only rarely being reported as an adverse event. In this case the rate of complaints with the ProVate Device is within the lower range of complaints cited in the literature,~~
* ~~Vaginal spotting is yet another anticipated AE, mainly when trying a new vaginal device. With ProVate Device there were 8 cases of spotting in 6 subjects (8.4%). There were 2 cases of Device discontinuation, in one (1) of them (#006-005, cited above) there were 5 recorded reasons for discontinuation without the ability to understand which of the AEs triggered discontinuation. In all other cases study ended as planned without treatment and with no residual effect. The reported rate of spotting in the literature is in the range of 6.9-47%~~~~[[90]](#endnote-90)~~~~,~~~~34~~~~. Therefore, rate cited here falls within the lower range of complaints cited within the literature.~~

**~~Non-anticipated AEs~~**

~~There were eight (8) non-anticipated, potentially device-related AEs in 4 subjects. 3 AEs were of abdominal cramps/menstrual like cramps in 3 different subjects, 1 case of lower back pains, and also one (1) case each of abdominal tenderness, urinary urgency, pelvic pressure and pelvic pain. All these AEs completely resolved, one (1) case (urinay urgency) was treated with Hydrochloride Phenazopyride, and one (1) subject (#006-005) discontinued the study after experienced 5 different AEs (please note table 13 below for listing).~~

### Vaginal Complaints Which May be Related to Vaginal Infections While Using ProVate or Control Devices

Failure criterion #2 required bothersome vaginal complaints, suggesting of vaginal infection, while failure criterion #3 also required treatment for a diagnosed vaginal infection

With the Control device, there were two (2) cases of bothersome vaginal complaints and one (1) case which required treatment for overt vaginal infection, in a subject who presented with bothersome malodorous vaginal discharge, during visit 7 (final visit). On examination she had a malodorous infectious discharge and inflammation of vaginal walls. She was treated with metronidazole.

There were no reported vaginal complaints potentially related to vaginal infections while using the ProVate Device.

### ~~Actions Taken Secondary to Adverse Events and Outcome~~

~~With ProVate Device:~~

* ~~In two (2) subjects (04-016, 04-023) device usage was delayed for a few days when they complained of vaginal discomfort while using the ProVate Device. There was no need for medical intervention. Following delay, further device usage was uneventful.~~
* ~~Four (4) subjects discontinued the study prematurely due to AEs which had reasonable causal relationship to the ProVate Device. Table 13 below summarizes the AEs which had reasonable causal relationship to the ProVate Device in the 4 subjects who prematurely terminated the study. As may be noted, these AEs were considered minor, mainly anticipated, and they all resolved spontaneously without medical intervention.~~
* ~~Medication treatment – Drug treatment was given to 2 subjects who had altogether 3 AEs. One (1) subject had urinary urgency and frequency (which were considered by the investigator as 2 different AEs) and was treated with Hydrochloride Phenazopyride, and one (1) subject had granulation tissue in the vaginal apex and was treated with Silver Nitrate application. These AEs resolved with only office-based medical interventions at at-home care.~~
* ~~Resolution of AEs - all AEs (100%, 40/40) considered as having reasonable causal relationship to the ProVate Device, resolved completely. Subjects did not have any complaint at the end of the device usage phase which could be attributed to the device.~~

~~Table 13-Discontinuation rate, AEs, and outcome in subjects who discontinued the study~~

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **~~Subject~~** | **~~AE~~** | **~~Severity~~** | **~~Anticipated~~** | **~~Outcome~~** |
| ~~01-016~~ | ~~Spotting~~ | ~~Mild~~ | ~~Yes~~  | ~~Recovered~~ |
| ~~02-007~~ | ~~Abdominal pains~~ | ~~Moderate~~ | ~~Yes~~  | ~~Recovered~~ |
| ~~06-005~~ | ~~Vaginal swelling~~~~Vaginal irritation~~~~Abdominal cramps~~~~Vaginal bleeding/Spotting~~~~Spotting~~ | ~~Moderate~~~~Moderate~~~~Moderate~~~~Moderate~~~~Mild~~ | ~~Yes~~~~Yes~~~~No~~~~Yes~~~~Yes~~ | ~~Recovered~~~~Recovered~~~~Recovered~~~~Recovered~~~~Recovered~~ |
| ~~06-006~~ | ~~Vaginal erosion~~~~Granulation tissue growth~~ | ~~Moderate~~~~Moderate~~ | ~~Yes~~~~Yes~~ | ~~Recovered~~~~Recovered~~ |

~~With Control Device:~~

~~3 subjects required medical treatment for AEs which had reasonable causal relationship to the device – in 2 cases for Urinary Tract Infection, and for treatment of overt vaginal infection in a subject who presented with bothersome malodorous vaginal discharge, during visit 7 (final visit). On examination she had a malodorous infectious discharge and inflammation of vaginal walls. She was treated with metronidazole.~~

~~88% (15/17) of AE's considered as having reasonable causal relationship to the Control Device, resolved.~~

~~In 2 cases AE’s continued after the end of the study, with need for further follow-up and decision making:~~

* + ~~De-Novo SUI in subject #04-022~~
	+ ~~Vaginal wall trauma in subject #06-021~~

### ~~Non Device Related Adverse Events~~

~~Table 14 below lists the non-device related AEs in the study.~~

~~Table 14-List of 28 Non-Device Related Adverse Events, further divided into Control and ProVate phases~~

~~(Safety population)~~

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **~~Non-Device Related Adverse Events~~**  | **~~Device~~** | **~~Seriousness~~** | **~~Intensity~~** | **~~Action taken~~** | **~~Treatment~~** | **~~Outcome~~** |
| ~~Bronchitis~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Upper Respiratory Tract Infection~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Tinea Pedis~~ | ~~Control~~ | ~~Not serious~~ | ~~Moderate~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Skin Irritation Left Cheek~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Flea bites in legs~~ | ~~Control~~ | ~~Not serious~~ | ~~Moderate~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Fatigue~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Gastric Ulcer~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~AE still present~~ |
| ~~Vaginal spotting~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Amebiasis~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~AE still present~~ |
| ~~Common Cold~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Dysmenorrhea~~ | ~~Control~~ | ~~Not serious~~  | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Hemorrhoidal pains~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Vulvar itch~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Ribs Fracture~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~AE still present~~ |
| ~~Unspecified pain left side~~ | ~~Control~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Migraine~~ | ~~ProVate~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Abdominal bloating~~ | ~~ProVate~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Foul vaginal odor~~ | ~~ProVate~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Vaginal discharge~~ | ~~ProVate~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Cough~~ | ~~ProVate~~ | ~~Not Serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Sore Throat~~ | ~~ProVate~~ | ~~Not Serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Cough~~ | ~~ProVate~~ | ~~Not Serious~~ | ~~Mild~~ | ~~Study discontinued~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Rectal bleeding~~ | ~~ProVate~~ | ~~Not Serious~~ | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Constipation~~ | ~~ProVate~~ | ~~Not Serious~~ | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Rt eye Blepharitis~~ | ~~ProVate~~ | ~~Not Serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Dysuria~~ | ~~ProVate~~ | ~~Not Serious~~ | ~~Mild~~ | ~~None~~ | ~~None~~ | ~~Recovered~~ |
| ~~Common cold~~ | ~~ProVate~~ | ~~Not Serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |
| ~~Common cold~~ | ~~ProVate~~ | ~~Not serious~~ | ~~Mild~~ | ~~None~~ | ~~Drug~~ | ~~Recovered~~ |

# Discussion

The PT-104 study was undertaken to evaluate the impact of the ProVate Device, intended for the temporary management of POP in women, on vaginal microflora while in use. The objective of the study was to confirm that the ProVate Device does not alter vaginal microflora in a clinically significant manner, as compared to a control (commercially available pessary). There were two (2) pivotal clinical investigations held with the ProVate Device for regulatory purposes – PT-103 study intended to demonstrate safety and effectiveness (ability to treat POP) and the current study which was limited to one safety aspect - impact on vaginal microflora.

## Data and Conclusions from the PT-104 Vaginal Microflora study

The study was designed as a multi-center, open label, prospective, randomized, controlled, statistically powered (non-inferiority), cross over, home-use study, conducted in seven (7) sites, in the US (63.8% of the study PP population) and in Israel (36.2% of the study PP population). Comparison was done between the ProVate Device and a US marketed vaginal ring pessary (Control).

Altogether, 383 fresh single-use ProVate Devices were used during the ProVate Device phase of the study (not including Devices that were used for short term at the clinic for sizing and training purposes). Most ProVate users used each Device between 4-8 days. Usages of Control Device were substantially longer, as expected.

Total use of devices across each usage phase was comparable between the ProVate Device and the Control device - cumulative mean ProVate Device total usage length, during the usage phase, per subject in the Per-protocol population, was 681.6 ±85.92 hours, (~ 28.4 ± 3.58 days), whereas mean cumulative Control device total usage length was 717.7 ±64.05 hours, (~ 29.9 ± 2.66 days).

### Meeting the Endpoint Criteria

The primary endpoint was based on failure criteria in order to demonstrate non-inferiority of the ProVate Device in comparison with the control. Failure was considered as meeting at least 1 failure parameter, out of the defined specific 3 failure parameters of the primary endpoint. Analysis of results showed that failure rate was the same with both devices – Nine (9) subjects (15.5% of the PP population) in each device group, with a 1-sided 97.5% upper limit of 13% which was within the non-inferiority margin of 15%. Hence, the primary endpoint was met successfully.

The secondary endpoints evaluated each of the failure parameters from which the primary endpoint is calculated, including the well-known fluctuations of the vaginal microflora. Results of specific item or microorganism, namely changes in Nugent’s score, levels of *Staphylococcus aureus* and Candida morphotypes, rate of bothersome vaginal symptoms and of symptoms which require treatment – were found to be comparable between ProVate and Control. Further, the study demonstrated lower rates than expected based on the literature (note section 3.4.3 above).

The ProVate Device is a disposable, single use, vaginal ring pessary designed to perform as the existing ring pessaries, hence it was not expected to be different from existing ring pessaries in respect of vaginal microflora changes, where changes occur spontaneously very often. Results from this study support this assumption where vaginal microflora was not affected by the ProVate Device any different compared to existing marketed vaginal pessaries.

Of note, patients use their existing reusable ring pessaries for years, and they are cleaned from time to time either by the user or at the clinic. Even when cleaned, this process typically only involves washing with soap and water, which may not fully remove microorganism growth from the device. In this PT-104 study, the design of the study required using a fresh new Control device, which was provided by the sponsor, in order to have the same device for all subjects, and therefore likely does not fully reflect the vaginal microflora during actual use after an extended period.

### Vaginal Microflora Normal Fluctuations

Vaginal microflora is unstable and continuous sampling may show marked variability in the presence and level of various vaginal microorganisms, at the same woman, even on a daily basis. This is true in both pre & post-menopausal women. Fluctuations of vaginal microflora have been extensively discussed in section 1.3.3 of this report, with a review of the literature.

The key message from the literature review is that fluctuations in vaginal bacterial communities are common, and that women may have short episodes of various adverse conditions, including significant changes in vaginal microflora, that spontaneously resolve without treatment. As guided by the CDC (and discussed above (note section 1.3.4), and by other clinical organizations outside the USA, these conditions do not necessitate any treatment, unless accompanied by specific vaginal complaints and / or symptoms such as vaginal thrush, pain, discomfort, infectious discharge, smell, etc.

Constant natural fluctuations in the vaginal microflora limit the value of laboratory results alone in the assessment of vaginal infections.

These fluctuations make studies like this very difficult to interpret as results of the microbial samples may change very often, regardless of the studied device. This may lead to wrong assumptions as to the presence or absence of various microorganisms. Therefore, in a clinical study, attention should be aimed at signs and symptoms of a possible vaginal infection rather than be limited to laboratory results, due to the expected microfloral fluctuations discussed above. Though numbers are very small and the difference is not significant – it is still interesting to show that in the PT-104 study, there were no signs of infection or bothersome complaints that required treatment with the ProVate Device – as opposed to 5 cases with the Control device. This may mean that the ProVate Device is at least as safe as the Control device.

### Vaginal Devices

Section 1.3.5 in this report brings an extensive discussion of vaginal devices and their role as possible causes of vaginal infections, with a review of the literature.

There are many vaginal devices in daily use, intended for various different uses (contraception, collection of blood, absorption of menstrual blood), made of different materials (plastics, silicone, nylon mesh, Rayon), and which remain in the vagina for various periods of time (ranging from 8 hours to several months). However, data gained so far and discussed above does not shows that vaginal devices, including the ProVate, cause adverse change of vaginal microflora, and does not support their role in promoting vaginal infections.

### Urogenital Infections

In many of the articles which discuss adverse events related to vaginal pessaries, one may find substantial amount of cases of vaginal infection or vaginitis. Alnaif & Drutz[[91]](#endnote-91) claimed that infection may affect as many as one third of pessary users. Bai et al[[92]](#endnote-92) claim that the rate of vaginal “inflammation” due to pessaries reaches 20% of users. Alperin et al[[93]](#endnote-93) found that 6% of pessary users had vaginitis (i.e. inflammation of the vagina) diagnosed within the first three (3) months after initial pessary placement, and that vaginitis was documented in 35% of patients through the nine (9) years follow-up period. Abdulaziz et al82 conducted a systematic review of the literature for adverse events while using vaginal pessaries. In a group of 394 women who used ring pessaries there were 31 cases (7.8%) of AEs described as ‘vaginal infection” or “vaginitis”.

Results from the PT-104 Microflora study, concerning urogenital infections, demonstrate very low rate of infections, as compared with the relevant literature.

* While using the ProVate Device, there were neither vaginal nor urinary infections (as concluded by the investigator) while using 383 Devices over 1647 usage days.
* While using the Control Device – there was one (1) case of overt vaginal infection which required treatment, and two (2) cases of UTI which also required treatment. Also, there were 5 cases with signs of infection or bothersome complaints that required treatment

In a previous study (PT-103 safety and efficacy study held with the ProVate Device) there were no vaginal infections at all while using 1592 ProVate Devices over 3558 usage days.

Results from this study and from the previous PT-103 safety and efficacy study support that vaginal devices do not promote vaginal infections (note discussion above, section 1.3.5. However we have no data comparing the use of a fresh-clean device at every insertion versus a device which is in constant use for years and is removed, cleaned, and immediately re-inserted, such as is the common practice for currently available pessaries. Since a large portion of pessary users cannot remove the ring pessary by themselves and need the help of a medical practitioner, removal and cleaning is performed every few weeks or even few months.

In the PT-104 study, a new clean Control device was dispensed to subjects, and was used by them, under stringent medical supervision, for only one (1) month. As discussed above, this does not necessarily reflect the long term use of current pessary devices and may have positively shifted the results in the case of the Control Device resulting in fewer infections or signs or symptoms of vaginal infections as compared with the data reported in the literature).

## ~~Ethnicity and possible different vaginal microflora~~

~~POP is ethnic dependent, where highest prevalence is among white women and lowest among African American women (note literature review in section 1.3). African American women are significantly less likely to report symptomatic prolapse, compared with white women, hence will probably seek treatment less frequently. This racial disparity in prevalence of subjective prolapse may be in part attributable to cultural attitudes toward the symptomatology of prolapse or its reporting.~~

~~This point of the rather low occurrence of symptomatic POP within the African-American population, and difficulty in recruiting African American women with symptomatic POP was previously discussed.~~

~~During interviewing of investigators for the PT-104 study, and during site selection, many of the clinics stated that African American women with POP of stages ≥2 are infrequently seen, that they hardly have any African American patients with a symptomatic prolapse which is also treated, and that these women are less likely to look for any treatment. Therefore, chances of recruiting African American women who are using a ring pessary for POP are rather low, even if recruitment is done outside of the clinics, by public advertisement.~~

~~The PT-104 study was conducted both in the US (37 subjects in the PP population), where there is a diversity of ethnicity, and in Israel (21 subjects). Only women with POP, who were also experienced with the use of vaginal ring pessary, were recruited into the study.~~

~~As all Israeli participants were considered white, ethnic distribution of the 37 US residents is as follows:~~

* ~~26 (70.2%) of subjects were non-Hispanic or Latino, 10 (27.0%) were Hispanic or Latino, and in 1 case (2.7%) data was missing (subject refused to discuss). US demographic statistics based on the US Census July 2018 population estimates state that ethnicity in the US is comprised of 81.9% non-Hispanic or Latino and 18.1% Hispanic or Latino.~~
* ~~32 (86.48%) of subjects were white, 2 (5.4%), were African-American, 2 (5.4%) were American Indian or Alaska Native, and 1 (2.7%) refused to discuss her race. USA demographics statistics state that 76.6% of US citizens are white, 13.4% are African American, 1.3% are American Indian or Alaska Native, 5.8% Asian and 0.2% Hawaiian.~~

~~In this rather small sample ethnicity is comparable to the cited US demographics.~~

## ~~Adverse Events~~

~~When a subject initially uses any type of a new intra-vaginal device, it is common that the first few days of use are accompanied by some discomfort and, occasionally, other mild adverse events during this initial learning and accommodation period. Complaints and AE’s, which are anticipated at that time of vaginal pessary accommodation or use, will usually include, discomfort, spotting, pain or some bleeding.~~~~Vaginal wall trauma is the most frequently reported complication of a pessary, presenting as foul odor, purulent discharge, irregular blood stained discharge, and increased vaginal fluid. It occurs in ~19.3% of pessary users (range 3-42.8%) and believed to be caused by pressure on vaginal walls and general presence of a device in the vagina during the initial accommodation period. Rate of vaginal wall trauma in the PT-104 Microflora study was low with the ProVate Device (5.6%) and within the lower range of regularly cited figures in the literature.~~

~~In most published studies addressing pessary usage, recording of AEs has been assessed at the end of the study only. In a study by Duenas, recording of AEs was done during the end-study meeting with the investigator[[94]](#endnote-94), where the rate of AEs among the studied population was 31.6%. Sarma (2009)~~~~88~~ ~~reported much higher incidence of specific AEs, such as bleeding with pessaries, in 46.8% of users.~~

~~In this PT-104 study, where stringent recording of AEs was done with a diary on a daily basis, during a weekly discussion with the study administrator and during the ongoing meetings with the investigator, the rate of AEs was 36.6% for ProVate and 34.4% for Control – comparable between devices and within the range of cited literature.~~

~~Altogether there were 57 AEs with reasonable causal relationship to the studied device in the study (Safety Analysis Population), in both the Device and the Control phases. 40 of those were with ProVate Device, out of which 34 (85%) were anticipated, and all AEs (100%) resolved.~~

~~17 AEs were with Control Device, where 10/17 were anticipated, and 15/17 resolved.~~

~~As expected, there were several important findings:~~

* ~~There were no serious device-related AEs, for either the ProVate or Control devices.~~
* ~~All AEs which had reasonable causal relationship to the device were minor. With the ProVate Device all AEs (40) resolved completely (100%), With Control device there were 17 AEs with reasonable causal relationship to the device, of which 88% resolved, however necessitating medications in 3 cases of urinary and vaginal infections.~~
* ~~There were no vaginal infections, or symptoms & signs of vaginal infection observed while using the ProVate Device.~~
* ~~Most of the ProVate device-related AE’s were anticipated (34/40, 85%) – namely vaginal spotting, vaginal discomfort, vaginal wall trauma, etc. With the Control device – less AEs were reported.~~
* ~~Altogether, for both ProVate and Control devices, rate of AEs with reasonable causal relationship to the device was low, within the lower range cited in the literature~~
* ~~In this study the total rate of all AEs for both ProVate and Control is low, and the rates for each device were lower than the rates reported in the literature. Explanations may include the following:~~
	+ ~~With Control device women did not need to perform their own insertions and removal which were all done for them at the clinic by a physician. The opposite will happen with any new device inserted into the body by the user where accommodation period is always accompanied by some degree of minor anticipated AEs, as discussed above.~~
	+ ~~Subjects enrolled in the study had all previously used pessaries before and thus were accustomed with the feeling of the commercially available ring pessary and therefore potentially did not report pain/discomfort/pressure within the vagina. Subjects using the ProVate Device reported higher level of discomfort than reported when using the Control Pessary, which may be at least partially attributable to an accommodation phase with a new device. However, the overall rates of vaginal discomfort reports were low. This did not impact the subject’s overall impression and satisfaction with use of the device.~~
	+ ~~The use of a fresh clean new Control device in the study, for only one (1) month duration, as opposed to using the subjects’ own regularly used reusable device for months at a time, may have positively affected the rate of infection-related AEs for the Control subjects compared to the experience they would otherwise have with their reusable device.~~

# Conclusions

The primary endpoint in this study was met successfully showing non-inferiority of the ProVate Device when compared to an existing market available ring pessary.

Results from this study show that with the ProVate Device, the rate of microfloral changes and vaginal complaints which are bothersome or requiring treatment is relatively low and comparable to an existing market available vaginal ring pessary. Adverse events related to the ProVate Device were minor, non-serious, and all resolved completely. Therewere no vaginal or urinary infections.

Taking the above mentioned low level of anticipated, mainly mild AEs which had reasonable causal relationship to the ProVate Device, together with the minimal microflora changes and absence of clinical vaginal infections, the ProVate Device demonstrates comparable minimal impact on microflora compared to the Control pessary with no additional safety concerns.

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