Multi-Gyn Luxembourg, Silvia Martakova, Mgr, 28, rue de Capellen, L-8279 Holzem
GSM: +352 661 666 878, e-mail: msa@msa-lux.lu, www.multi-gyn.lu

STUDY

Study Report

Relief and prevention of vaginal complaints in (post) menopausal women

BIOCLIN CONFIDENTIAL INFORMATION
Document: MG-Study- Report Relief and prevention of vaginal complaints in (post) menopausal women
Updated: April 2013
By: Lisanne Ranzijn

Relief and prevention of vaginal complaints in (post) menopausal women

Content

Relief and prevention of vaginal complaints in (post) menopausal women

1. Introduction

Urogenital symptoms associated with estrogen loss can occur episodically throughout a woman's life (e.g. during lactation, during treatment with GnRH agonists, etc.) but it is most common and chronic in duration in postmenopausal women. Complaints associated with urogenital ageing include vaginal dryness, irritation and pressure, vaginal discharge and infection, vulvo-vaginal pruritis, dyspareunia, post-coital bleeding, urinary frequency, urgency and incontinence and recurrent urinary tract infections. Although these symptoms have affected women for centuries, they are now becoming more widely recognized by health professionals and society in general because of the increased life expectancy.

2. Bacterial vaginal flora in (post)menopausal women

Estrogen levels regulate the thickness of the mucous epithelial lining of the vagina. Lack of estrogen results in atrophy of these tissues. Lactobacilli, yeasts, and bacterial vaginosis-associated bacteria are less commonly part of the vaginal microflora in postmenopausal women than in women of reproductive age. High numbers of peri- and postmenopausal women have no lactobacilli and no bacterial-vaginosis-associated microorganisms. The literature on the incidence of the disruption of the vaginal flora of postmenopausal women shows different results.

Certain studies show that the physiological reduction in lactobacillus colonization of the vagina in postmenopausal women does not seem to cause an increase in bacterial vaginosis prevalence). It was shown that “of 100 women examined, 44 had grade 1 flora, 17 had grade 2 flora and 18 had BV. An apparent absence of, or very scanty, vaginal bacteria in which grading was not possible was found in 21 women. Women with vaginal atrophy were more likely to have an apparent absence of vaginal bacteria, but a few had BV. Women on estroprogestinic hormone replacement therapy (HRT) showed to be slightly less positive for bacterial vaginosis .

Elsewhere it is noted that normal biocenosis of the vagina was found in 42.27% and that the other part of the study group had abnormal vaginal biocenosis or had no microorganisms at all. During the hormonal therapy, the group of women with normal biocenosis increased to 53.65% and 62.29% after respectively 3 and 9 months of therapy. Hormonal therapy secured continuity of normal Lactobacillus morphotypes.

A recent study however study shows that 70% had either intermediate-grade bacterial colonization or bacterial vaginosis (BV) and concludes that “asymptomatic BV appears to be much more common than is currently perceived” and that “these findings may have implications for the health of postmenopausal women”.

Among postmenopausal women attending a vaginitis clinic, a defined diagnosis of bacterial vaginosis, C. albicans or T. vaginalis infection could be made in about one third of such clients. Concerning the two thirds of symptomatic women lacking such a microbiologic diagnosis, alternative causes (e.g., estrogen deficiency, nonanaerobic bacterial infections, local irritants or allergenes, and dermatologic conditions) need to be considered.

With regards to candidiasis; symptomatic vulvovaginal candidiasis is rare in postmenopausal subjects because of the estrogen-dependence of this infection. Also diabetes seems to be a predisposing factor. Among non-diabetic postmenopausal females, C. albicans adherence was lower than for fecund controls, but it was higher for cells from postmenopausal diabetic women.

3. Vaginal atrophy in (post)menopausal women

After the menopause decreased concentrations of oestrogen may result in insufficient maturation of the vaginal epithelium, which can lead to a range of vaginal discomforts. More than 50% of postmenopausal women experience lack of vaginal lubrication and have frequent vaginal infections.

4. Rationale for the study

In the present "Placebo"-controlled study we intend to research in (post)menopausal women with the regular topical application of a bio-degradable bio-adhesive acid gel in which the Verum comprises an extra component that inhibits adhesion of pathogens. It should be noted that ""Placebo"" is not the correct description of the control gel; the bio-adhesive thickener and the pH are essential parts of the formula of Multi-Gyn Actigel and part of its registration as a Medical Device Class 2A.

The lack of lubrication by the atrophic vaginal tissues not only makes sexual intercourse unpleasant but also often results in friction damage by sexual intercourse, makes the tissues more vulnerable to microbial invasion or infectious processes. 53% of the post-menopausal women show signs of genital atrophy.

The rationale of this study was the prevention of microbial invasion, particularly that of coccoid bacteria that are related to the clinical signs of Bacterial Vaginosis, during and after menopause by the manipulation of the vaginal environment. This is partly achieved with the regular topical administration of a vaginal gel that installs the optimal vaginal pH of 4 –4.5. This pH prevents adhesion of pathogenic microorganisms. Vaginal discomforts that are related to BV related microbial disbalance may disappear as a result.

In this "Placebo" controlled study the possible added value of the addition of the manipulated Aloe barbadensis extract with the patented anti-adhesive compound 2QR in the Study Gel Verum was compared with the gel without this compound. The manipulated Aloe Barbadensis extract has an elevated percentage of polysaccharides, specifically of a negatively charged polysaccharide "Galactoarabinan Polyglucuronic Acid Crosspolymer" (2QR) that inhibits adhesion of pathogenic microorganisms and that appears to have also strong itch stopping properties. Aloe Barbadensis extract also simulates growth of lactobacilli.

Both study gels, Verum and "Placebo", had the pH 4.1 of the natural anti-microbial defence of the optimal vaginal environment. Both the "Placebo" and the Verum had the same bio-adhesive thickener. The thickener is a natural biodegradable gum. The efficacy of such a preventive regime was statistically evaluated for the frequency of vaginal complaints and infections -particularly those related to BV.

In this study a large number of women with vaginal atrophy was expected. Lubrication and moisturizing with a non bio-degradable thickener could be effective in relieving symptoms related to vaginal atrophy. The study rationale therefore included the assessment of the improvement of the status of the vaginal tissues with the observation of the atrophic cytology in the vaginal smears and the improvement of the complaints thereof as induced with the lubrication of the vaginal tissues by means of the strongly bio-adhesive thickener of as well the "Placebo" as the Verum Gel.

With the comparison between the acid gel base of Multi-Gyn Actigel –Study Gel "Placebo"- and Multi-Gyn Actigel with the specific 2QR polysaccharide complex –Study Gel Verum we wanted to evaluate the added value of 2QR in the efficacy of the results between Verum and "Placebo" in:

- the frequency of complaints during 6 month application as compared to the clients history of complaints
- the treatment of the complaints at study entry with a one week treatment course
- the effect on the vaginal flora after one week treatment
- the recurrence of the complaints of study entry during the 6 months of the study
- the severity of the complaints of study entry and during the 6 months of the study
- the status of the vaginal flora during the 6 months of the study
- the status of the vaginal tissues during the 6 months of the study

4.1 Material and Methods

All study materials were provided by BioClin. The study design was open label "Placebo"-controlled. Eligible clients were randomized upon their entry number in the investigators premises. Odd client numbers received Study Gel O and even client numbers received Study Gel E. Study Gel O was the Verum with the 2QR compound and Study Gel E was the "Placebo" without 2QR. Both products were otherwise identical and had the pH 4.1 of the optimal vaginal environment and the same thickener that adheres well to mucous tissues.

The selection criteria were primarily:

a) a vaginal complaint for which medication is not imperative
b) expected compliance
c) the mental capability to follow the instructions of the study during the full course of the study
d) clients on Hormone Replacement Therapy (HRT) were to be excluded
e) clients on topical vaginal estrogen preparations were to be excluded

Otherwise there were no restrictions with respect to physical or social conditions.
Upon their first presentation to the investigator for a vaginal complaint, the investigator explained the client the aim of the study and asked the client to participate.

The parameters for the study evaluation were:

- the results of the cytological evaluation of the smears during the course of the study
- the frequency of vaginal complaints during the course of the study period
- the number of Interim Visits
- the frequency of required medication for the treatment of vaginal complaints
- the severity of vaginal complaints as reported by the client (itch, pain, fluor etc.)
- the status of the atrophy, when present

BioClin provided a draft of the Client Information, Instruction Leaflet and Client Diaries which have been translated into Serbian language for distribution to the clients. The Client Diaries comprised the comments of the client relevant to vaginal complaints, discomfort or comfort and concomitant situations such as disease, sexual intercourse, stress etc. The investigator collected the Client Diaries and transcribed the information unto the Clinical Research Forms. Any concomitant medication such as a course of antibiotics was also documented.

The Clinical Research Forms comprised the translated items of the Client Diaries, the observation data of the investigator relevant to the vaginal condition of the client and the results of the cytological evaluation of the Client Test Smears.

BioClin provided a Fluor Test Kit consisting of slides, sampling brushes, pH strips, a fast staining fluid and KOH solution for the cytological evaluation of the smears by the investigator. The smears were collected by the monitor and sent to Dr. Mathilde Boon of the Leiden Cytology and Pathology Laboratory (LCPL) for final evaluation. This evaluation was taken as parameter in the study.

Visit 1

Clients who were included in the study during a first visit to the investigator had the first vaginal smear cytologically evaluated, pH value registered and the severity of the complaint indicated during that visit. Information relevant for the study was collected and noted on the CRF (Clinical research Form) of Visit 1.

The client received all explanations and instructions on the application of the Study Gel as well as the completion of the Client Diary. Clients were specifically instructed not to use any product without advise/prescription of the investigator during this study. The client received the Study Gel O or E according to her Odd or Even client number. An appointment was made after a 5 days treatment course of the application of at least 2 x per day of the Study Gel O or E.

Treatment Visit 2

On the CRF of Treatment Visit 2 the results were noted as well of the cytology as well of the indicated severity of the complaints. During this visit the client received sufficient Study Gel for a period of 3 months regular application of the gel. The frequency of application during the course of the study as a preventive measure was every other day; in the evening before going to sleep.

An appointment was made for the Half Term Visit 3 (3 months). Clients were also instructed to make an interim appointment with the investigator when any vaginal discomfort such as itch, excessive discharge, unpleasant smell of the discharge, burning etc. occurred. In that case, when no medication was imperative, a treatment of 5 days was again initiated with increased application of the Study Gel; at least 2 x per day. The client was asked to return again after 5 days treatment for control.

Half Term Visit 3

Client test smear was conducted and the information of the Client Diary Forms transferred into the CRF by the investigator.

End of Study Visit

Client test smear was conducted and the information of the Client Diary Forms transferred into the CRF by the investigator. The preventive results of the study were evaluated. The End of Study Form was completed.

4.2 STUDY EVALUATION

The CRF's of the client were collected by the monitor. The smears were collected for expedition and evaluation by the LCPL. The original CRF's were sent to BioClin for evaluation by the statistician. Copies of the CRF's are kept with the investigator.

4.3 STUDY DURATION AND NUMBER OF CLIENTS

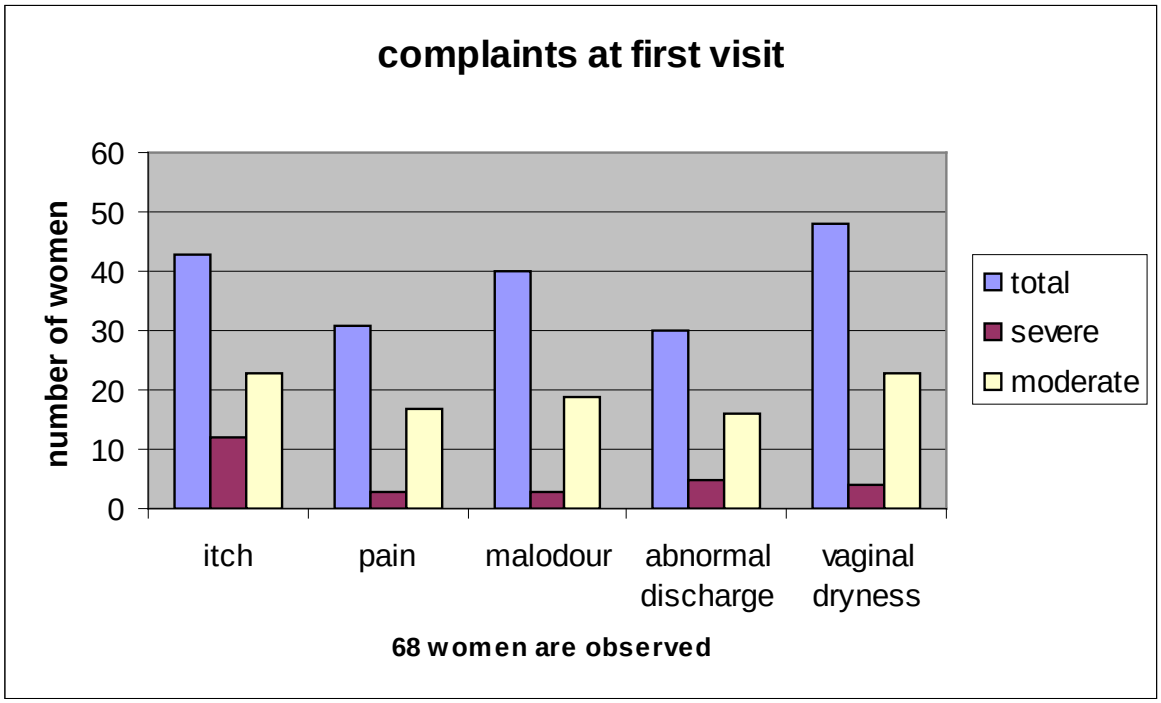
The study duration was determined by the inclusion of 50 participants or any surpassing number of participants in a period of 1 year. In the period of one year 60 clients finished the study for evaluation; 30 in the Verum and 30 in the "Placebo" group. At the 1 week treatment visit 68 clients could be evaluated.

5. STUDY RESULTS

5.1 COMPLAINTS

At study entry 68 women were observed. Vaginal dryness was the most reported complaint (48 women), followed by itch (43 women), malodour (40 women), pain (31 (women) and abnormal discharge (30 women). *See table 1*

Table 1



5.1.1 Vaginal dryness

For the complaint vaginal dryness 25 women were in the Verum group and 23 in the "Placebo" group. After one week treatment with Verum 9 women still complained of vaginal dryness versus 12 in the "Placebo" group. After 3 months of every other day application of gel this number did not change significantly. However after 6 months only 2 women in the Verum group versus 8 women in the "Placebo" group still had a complaint of vaginal dryness. The decrease of the severity after the one week treatment was greater in the Verum group (65 %) versus "Placebo" group (50 %). This decrease continued during the 6 month maintenance application for the Verum group to 92 % and for the "Placebo" group 68 %.
See tables 2 and 3

Table 2

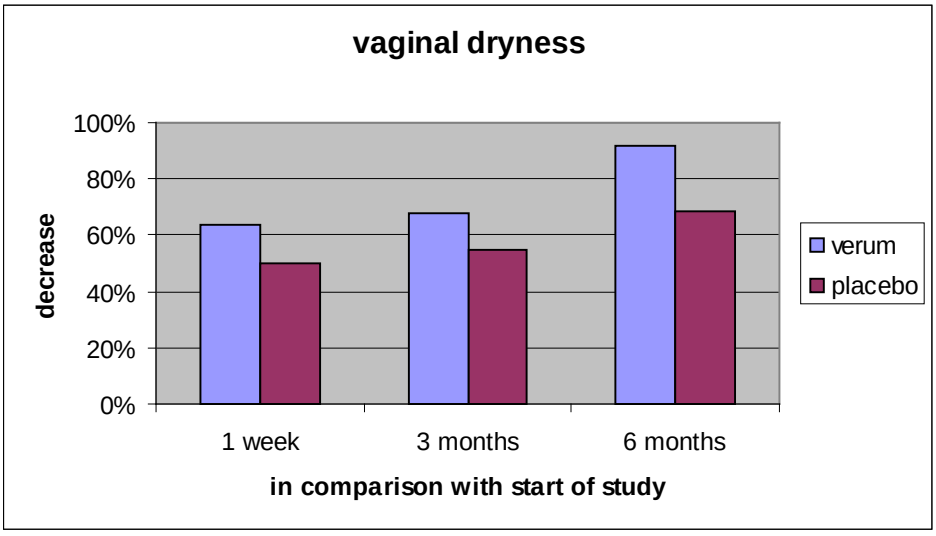
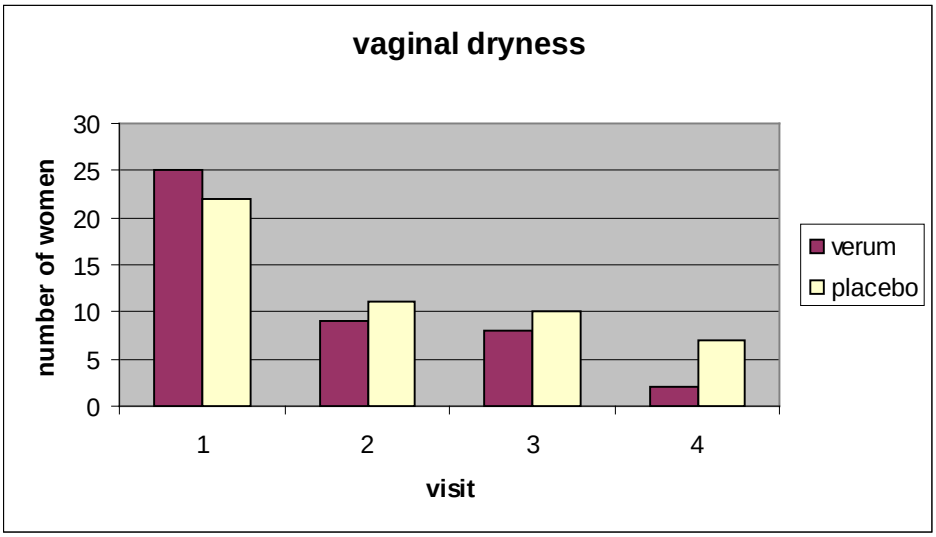


Table 3



Percentages of Decrease of Vaginal Dryness

	verum	placebo	Added value of 2QR in verum
1 week	64%	50%	verum has a 28 % greater effect
3 months	68%	55%	verum has a 24 % greater effect
6 months	92%	68%	verum has a 35 % greater effect

5.1.2 Itch

The decrease of the number of women who had the complaint itch was after one week treatment slightly greater in the Verum group (29 %) versus "Placebo" group (20 %), which still means that Verum had a 30 % greater effect than "Placebo". This decrease continued during the 6 month maintenance application for the Verum group to 72 % and for the "Placebo" group 56 %. *See tables 4 and 5; number of women with the complaint "itch"*

Table 4

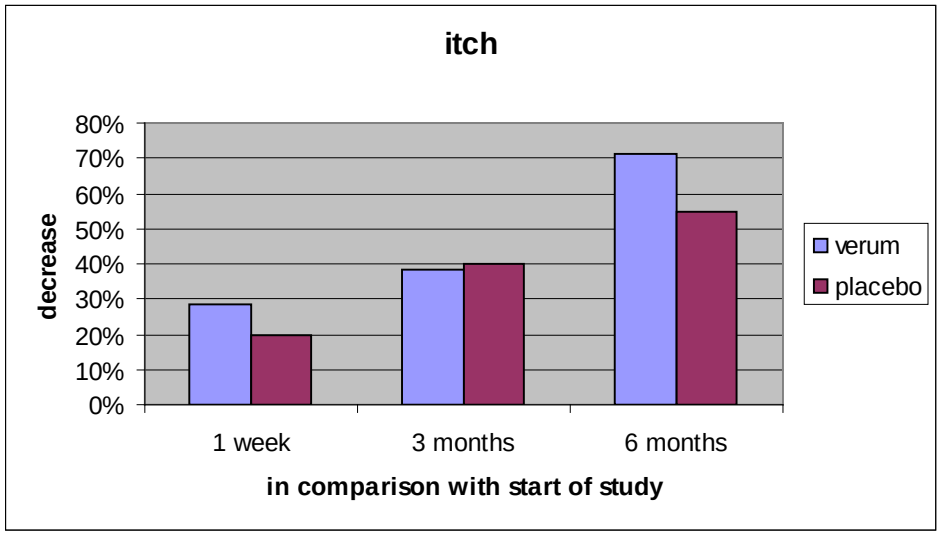
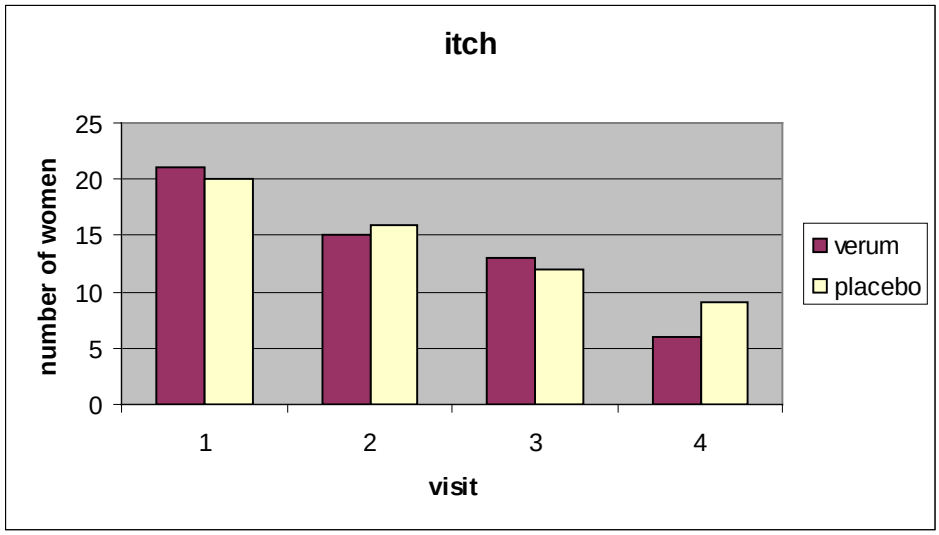


Table 5



Percentages of Decrease of Itch

	verum	placebo	Added value of 2QR in verum
1 week	29%	20%	verum has a 45 % greater effect
3 months	38%	40%	verum has an equal effect
6 months	71%	55%	verum has a 29 % greater effect

5.1.3 Malodour

The decrease of the severity of malodour after one week treatment was greater in the Verum group (45 %) versus "Placebo" group (25 %). This decrease continued during the 6 month maintenance application for the Verum group to 72 % and for the "Placebo" group 63 %.
See table 6 and 7

Table 6

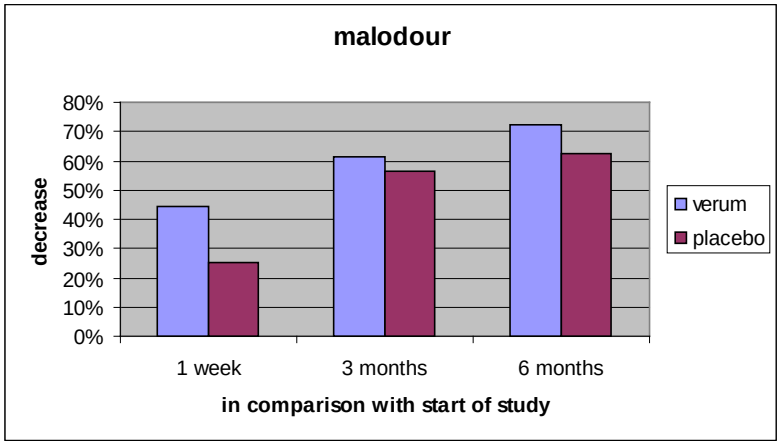
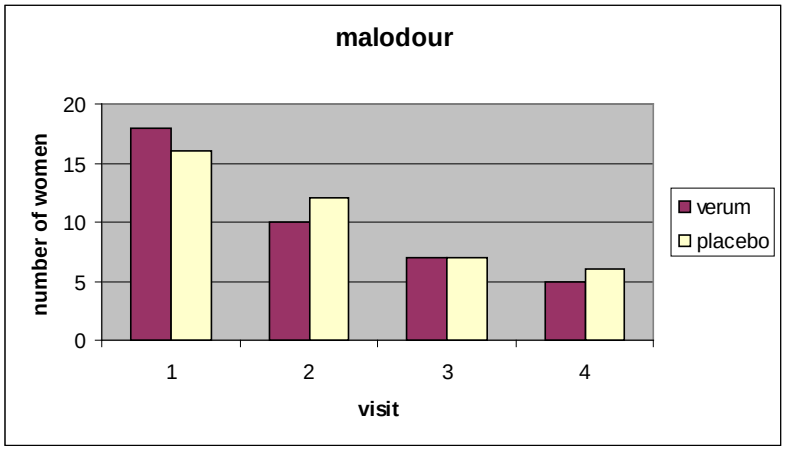


Table 7



Percentages of Decrease of Malodour

	verum	placebo	Added value of 2QR in verum
1 week	44%	25%	verum has a 76 % greater effect
3 months	61%	56%	verum has a slightly greater effect
6 months	72%	63%	verum has a 14 % greater effect

5.1.4 Pain

The decrease of the severity of pain after one week treatment was greater in the Verum group (40 %) versus "Placebo" group (25 %). This decrease continued during the 6 month maintenance application for the Verum group to 87 % and for the "Placebo" group 69 %. *See table 8 and 9.*

Table 8

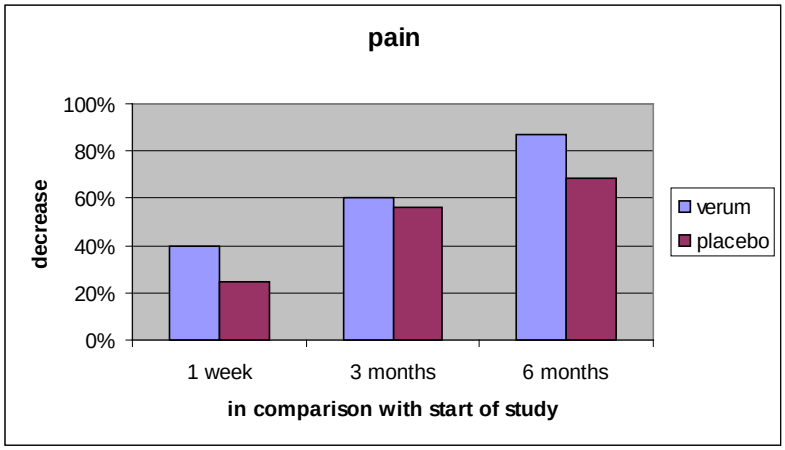
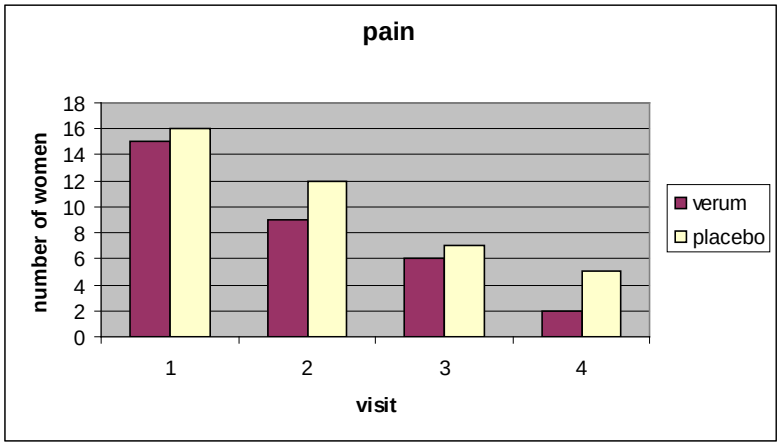


Table 9



Percentages of Decrease of Pain

	verum	placebo	Added value of 2QR in verum
1 week	40%	25%	verum has a 60 % greater effect
3 months	60%	56%	verum has an equal effect
6 months	87%	69%	verum has a 26 % greater effect

5.1.5 Abnormal discharge

The decrease of the severity of abnormal discharge after one week treatment was greater in the Verum group (65 %) versus "Placebo" group (50 %). This decrease continued during the 6 month maintenance application for the Verum group to 92 % and for the "Placebo" group 68 %. *See table 10 and 11*

Table 10

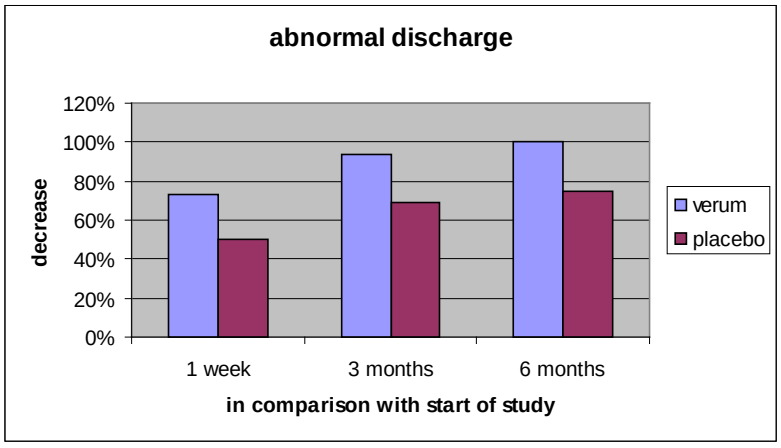
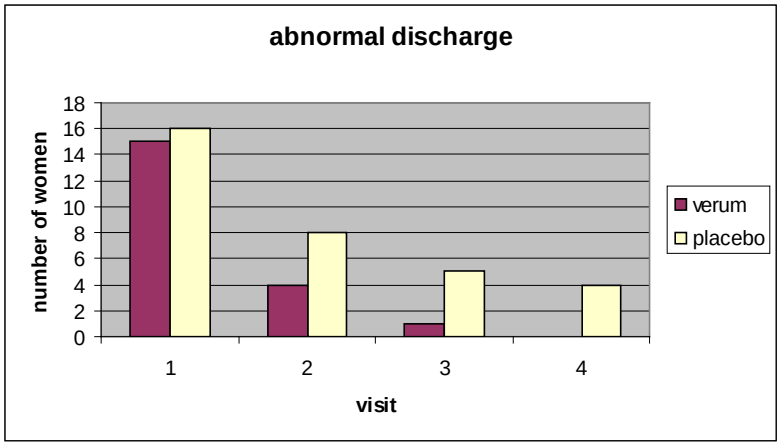


Table 11



Percentages of Decrease of Abnormal Discharge

	verum	placebo	Added value of 2QR in verum
1 week	73%	50%	verum has a 46 % greater effect
3 months	93%	69%	verum has a 35 % greater effect
6 months	100%	75%	verum has a 33 % greater effect

5.2 Cytology

As we have concluded in previous studies the cytology of vaginal smears most often does not reflect the presence of complaints nor the severity of complaints.

5.2.1 Bacteria

In this (post) menopausal group of women that participated in the study because of a vaginal complaint at study entry, a high percentage of bacterial vaginosis and dysbacteriosis could be expected as reported in other studies in this group of women. In a recent survey of smears of this age group of women in the Netherlands almost 40 % showed dysbacteriosis. However, in this Serbian population of Belgrade and its vicinity only in 4 smears coccoid bacteria could be detected in the 60 clients of this study. However lactobacilli were seen in the smears of 50

women. In 6 women no bacteria were present in the smears. Candida was not seen at all. No clinical nor statistically evaluable information can be drawn from the bacteriological observations in the smears of this group of 60 women. *See table 12 and 13*

Table 12

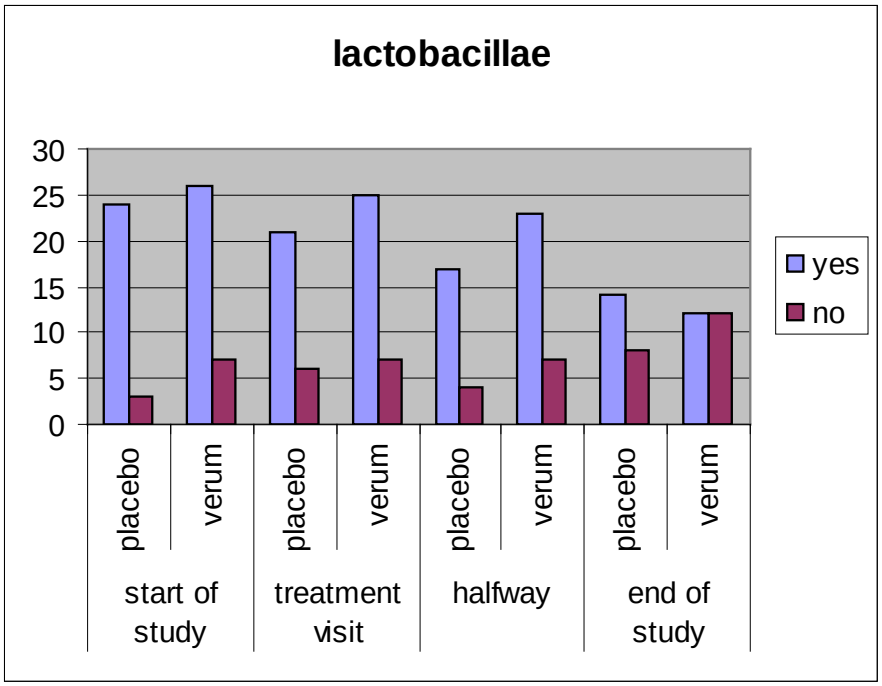
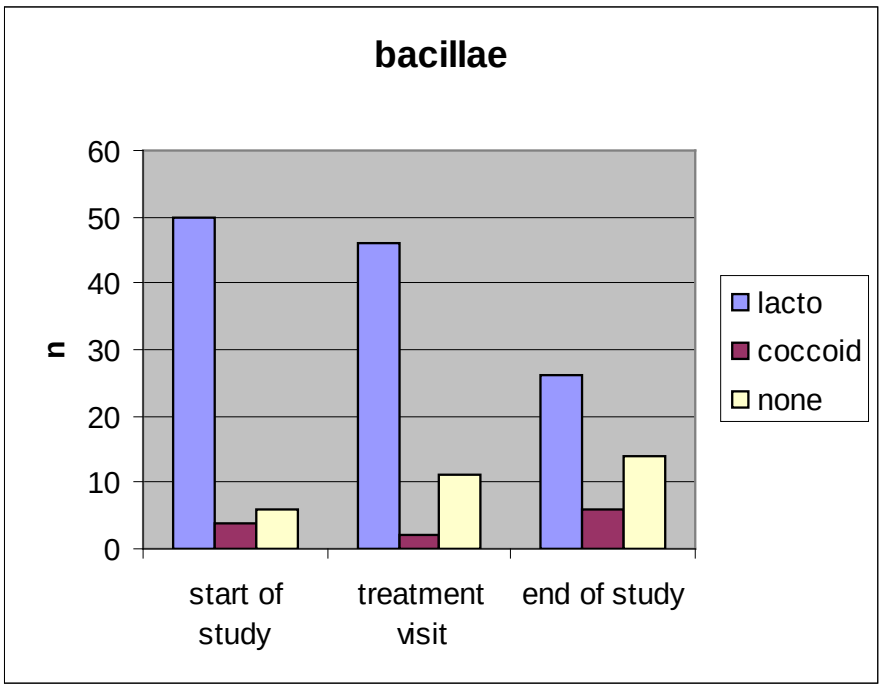


Table 13



5.2.2 Inflammation (granulocytes)

In 11 smears inflammation cells were observed. During the course of the 6 month study we saw no difference in this number. In the study group of 60 women a minor relationship between the presence of granulocytes and discharge could be demonstrated but none with any of the other complaints. The number is however too small to present relevant information. *See table 14 and 15*

Table 14

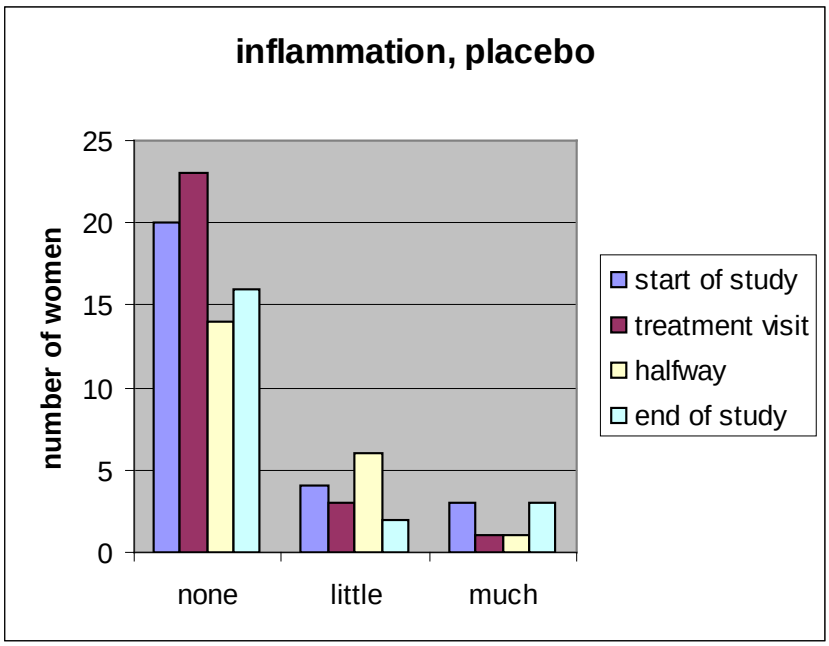
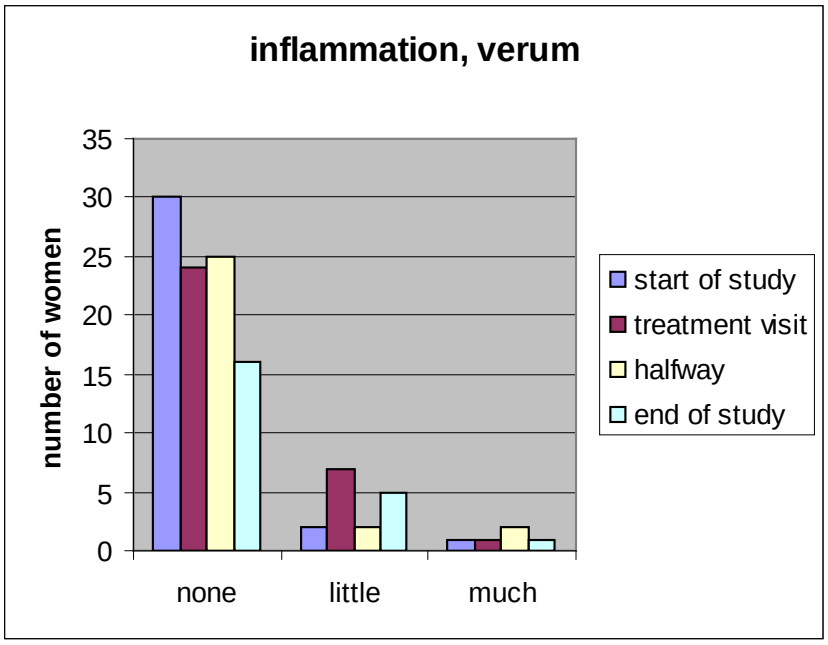


Table 15



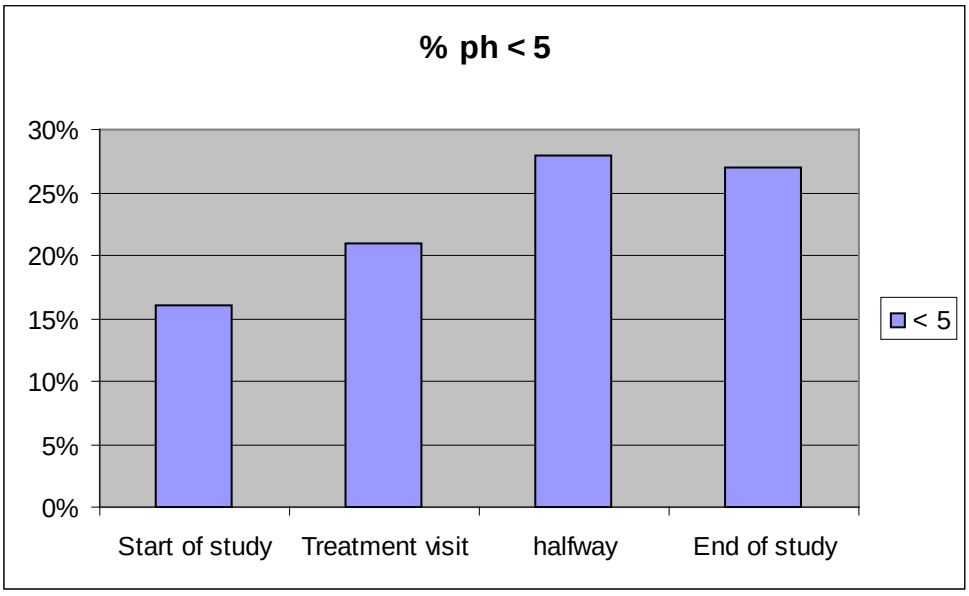
5.2.3 Atrophy

In this study of 60 women in the Serbian population of Belgrade and its vicinity in 39 smears atrophy was diagnosed; in 16 little, in 11 moderate and in 12 severe atrophy. It was expected that particularly the most reported complaints of vaginal dryness and also of pain and itch would show a relationship to atrophy in the cytological evaluation. However, no correlation with any of the vaginal complaints could be demonstrated in this group of women.

5.3 pH

During the 6 months study period the mean pH of all clients in the study decreases slightly. The percentage of women with a low pH < 5 goes up until the 3 months visit and is stabilized at the 6 months visit. *See table 16*

Table 16



5.4 Sexual activity

Because we assumed that the presence of vaginal complaints would influence sexual activity this parameter was included. It should be noted that not all women felt comfortable to report on this subject. No sexual activity was reported by 20 women and this did not change during the 6 months of the study. No difference in sexual activity was reported by 18 women. Increased sexual activity was reported by 22 women.

5.5 Interim visits

One patient came back for an Interim Visit in the first 3 months, because of severe inflammation and severe atrophy. After this second treatment the inflammation was milder. The client continued again with the maintenance schedule of every other day application. At the half term visit the inflammation had disappeared. The atrophy had also diminished. At the end of study there was no inflammation and mild atrophy.

5.6 Evaluation of the study gels

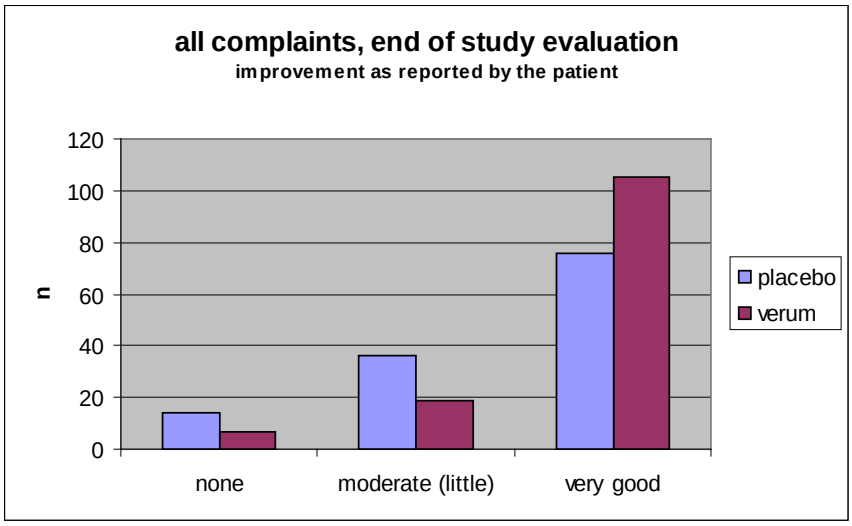
In the End of Study Evaluation the investigators as well as clients reported on the results of the study gels. The physician gave the opinion on the bacteriology and the physical effects and the clients gave her opinion on her physical experience. It is quite remarkable that in all major complaints the Verum received a higher approval rate than the "Placebo".

MD reports

		study gel		Total
		placebo	verum	
evaluation for bacteriology	none	6	6	12
	moderate (little)	12	11	23
	very good	8	11	19
Total		26	28	54

		study gel		Total
		placebo	verum	
evaluation for atrophy	none	3	2	5
	moderate (little)	11	8	19
	very good	12	18	30
Total		26	28	54

Patient reports



6. Discussion

The rationale for this study was to evaluate the added value of 2QR in Multi-Gyn Actigel in the clinical efficacy of the relief and prevention of vaginal complaints in (post) menopausal women when compared to its "Placebo". The "Placebo" is an identical gel without the 2QR compound and it should be noted that the bio-adhesive thickener is an essential part of the formulation.

As could be expected the control of the vaginal pH and the bio-adhesive thickener of the "Placebo" gel have a beneficial effect with both study gels. It was however shown that the presence of the 2QR component super-imposes all other beneficial effect. For all major complaints the Verum had a stronger clinical results as well after one week treatment as after 6 months maintenance application.

The results are in concordance with the in vitro test results which showed the added value of the 2QR component in a pH neutral test model versus pH 4.1. In this test model the effect on various vaginal flora was researched. It could be concluded that the pH neutral gel had a major capacity to control bacterial growth and that the low pH of Multi-Gyn Actigel has a super-imposing effect. In other words; the pH is certainly an important factor in the management of the vaginal environment, but the 2QR component has a major added value.

With regards to the cytology of the smears it was surprising to find few bacteria or inflammatory cells in the smears of this number of patients in this Serbian population. Therefore no statistically relevant comparison could be made between the Verum and "Placebo" for the effect on the vaginal flora. The non-relationship between a vaginal complaint and the vaginal cytology is evident and supports previous observations.

It was also surprising to find no relationship between atrophy and any of the vaginal complaints in this study group. Therefore the possible effect of the study gels on atrophy could not be statistically evaluated.

The increase of sexual activity showed a clear correlation with the decrease of vaginal complaints

7. Conclusion

The results of this open label "Placebo" controlled study with Multi-Gyn Actigel on relief and prevention of vaginal complaints in (post) menopausal women in Serbia it can be concluded that an acid gel is beneficial but that the 2QR component in Multi-Gyn Actigel has a strong added value in the relief and prevention of all frequently occurring vaginal complaints. The added value was great (28-76 %) for the one week treatment and considerable (14- 35 %) after the 6 months maintenance regime.

8. SERUM TESTS

Peptides (blood, serum, pus) have a neutralising effect; the efficacy of anti-microbial substances decreases and can even disappear completely. With the addition of serum albumin to a sample of Multi-Gyn ActiGel a worst-worst situation is created which is comparable to a severe infection of the vaginal mucous tissues which is expressed by excessive discharge. This means that also the germ killing components of a product such as Multi-Gyn ActiGel will have difficulty in killing the strains in a Challenge Test. The strains used in the Challenge Test were "wild" strains. The selection of microorganisms relevant for the vaginal flora consisted of a Lactobacilli mixed flora, Candida albicans, aerobic and anaerobic bacteria from human faeces.

To evaluate the influence of the pH of Multi-Gyn ActiGel on the test results a sample of a pH neutral (pH 6.5) Multi-Gyn ActiGel was submitted as well. The pH value of Multi-Gyn ActiGel is synergistic in its activity against microorganisms. The mayor part of this activity is derived from the main component of Multi-Gyn ActiGel; the bio-active polysaccharides of the 2QR complex. The results of this modified Challenge Test model with the addition of human serum to Multi-Gyn ActiGel are very promising for in vivo situations.

Multi-Gyn ActiGel pH neutral + 10% human serum*

	at 0 h	after 2 d	after 7 d
date	14-10-03	16-10-03	21-10-03
Lactobacillus mixed population	1,2X10.6	1,0X10.6	1,0X10.6
Candida albicans	1,2X10.6	1,0X10.2	< 10
aerobic bacteria from hfs [4]	2,2X10.9	4,0X10.6	1,0X10.6
anaerobic bacteria from hfs [4]	1,5X10.10	1,0X10.5	< 10

Multi-Gyn ActiGel pH 4.1 + 10% human serum *

	at 0 h	after 2 d	after 7 d
date	14-10-03	16-10-03	21-10-03
Lactobacillus mixed population	1,0X10.6	1,2X10.6	1,2X10.6
Candida albicans	1,2X10.6	< 10	< 10
aerobic bacteria from hfs [4]	2,X10.9	5,0X10.4	< 10
anaerobic bacteria from hfs [4]	1,0X10.10	1,0X10.5	< 10

* to simulate in vivo clinical conditions associated with BV.

Investigators

Dr. Boris Divic
Ul. Branka Radicevica 6
SCG-22000 Sremska Mitrovica, Serbia

Dr. Marta Savic
Branicevska 6
Gyn. Ordinacija Sretenovic
11000 Belgrade, Serbia

Dr. Zoran Sretenovic
Branicevska 6
Gyn. Ordinacija Sretenovic
11000 Belgrade, Serbia

Dr. Marjan Huljic
Gynecologist Dom Zdravlja Novi Beograd
Nedeljka Cabrinovica 64
11000 Belgrade, Serbia

Sanja Djurovic
Gradska Bolnica
Rifata Burdzevica 31,
11000 Belgrade, Serbia

Gordana Obradovic-Hajdukovic
Gradska Bolnica
RifataBurdyevica 31,
11000 Belgrade, Serbia

Cytology

Leiden Cytology and Pathology Laboratory (LCPL)
Rooseveltlaan 4 C
2321 BM Leiden, The Netherlands

Statistics

Dr. Matthijs Boon,
Leiden Cytology and Pathology Laboratory (LCPL)
Rooseveltlaan 4 C
2321 BM Leiden, The Netherlands

Sponsor

BioClin B.V.
PO Box 95
2600 AB, Delft, The Netherlands