Clinical Study Protocol

A double-blinded, randomized, placebo-controlled, parallel-group study evaluating the effect of the probiotic *Lactobacillus reuteri* on recurrent urinary tract infection (UTI) in adult women recently treated for UTI.

Sponsors study code: CSUB0144

NCT 03366077

Document Date: 24 Oct 2017

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Clinical Study Protocol	
Test Product	*Lactobacillus reuteri*
Study code	BioGaia CSUB 0144
Protocol Version and date	Final; V.1.0, 2017-10-24

STUDY TITLE

A double-blinded, randomized, placebo-controlled, parallel-group study evaluating the effect of the probiotic *Lactobacillus reuteri* on recurrent urinary tract infection (UTI) in adult women recently treated for UTI.

Test product and dosage	Once daily oral administration of a combination of two *L. reuteri* strains (DSM 16666 and DSM 17938)
Comparator product and dosage	Placebo, once daily oral administration
Duration of intervention	Three months (90 days)
Sponsor signatory	Kerstin Nilsson BioGaia AB Kungsbroplan 3 SE-112 27 Stockholm, Sweden Phone: +46 73 439 7080 E-mail: kn@biogaia.se
Principal Investigator	Erik Rein-Hedin Phone: +46 70 755 20 89 E-mail: erik.hedin@ctc-ab.se
Clinical study conduct and management	CTC Clinical Trial Consultants AB Dag Hammarskjölds väg 13 SE-752 37 Uppsala, Sweden

The following amendments have been made to the Final Clinical Study Protocol Version 1.0:

Amendment No.	**Date of Amendment**	**Revised protocol version**
Not applicable	*Not applicable*	*Not applicable*

2 STUDY SYNOPSIS

Study Title

A double-blinded, randomized, placebo-controlled, parallel-group study evaluating the effect of the probiotic *Lactobacillus reuteri* on recurrent urinary tract infection (UTI) in adult women recently treated for UTI.

Study code

BioGaia CSUB 0144

Study period

Estimated date of first subject enrolled: Q4 2017

Estimated date of last subject completed: Q4 2018

Principal Investigator

Erik Rein-Hedin, CTC Clinical Trial Consultants AB (CTC AB), Uppsala, Sweden

Study design

A double-blinded, randomized, placebo-controlled, parallel-group study.

Objectives

Primary objective

The primary objective is to evaluate the effect of oral supplementation with *Lactobacillus reuteri* on the frequency of confirmed UTI during six months from start of intervention in adult women with recurrent UTI.

Primary endpoint

Mean number of confirmed UTIs during six months from start of intervention, as compared to placebo.

Secondary objectives

1. To evaluate the effect of oral supplementation with *Lactobacillus reuteri* on the frequency of confirmed UTI during the three months´ intervention period and during the three months' follow-up period, respectively.
2. To evaluate the effect of oral supplementation with *Lactobacillus reuteri* on time to first relapse of UTI.
3. To evaluate the effect of oral supplementation with *Lactobacillus reuteri* on relapse rate at three and six months after start of intervention.
4. To assess the tolerability of oral supplementation with *Lactobacillus reuteri* in adult women with recurrent UTI.

Secondary endpoints

- Mean number of confirmed UTIs during the three months' intervention period, as compared to placebo.
- Mean number of confirmed UTIs during the three months' follow-up period, as compared to placebo.
- Mean time from start of intervention to first relapse of UTI, as compared to placebo.

- Proportion of subjects (%) who experienced at least one confirmed UTI during three months from start of intervention, as compared to placebo.
- Proportion of subjects (%) who experienced at least one confirmed UTI during six months from start of intervention, as compared to placebo.
- Occurrence and frequency of Adverse Events (AEs).

Number of subjects planned

A total of 140 subjects completed will be required, 70 in each intervention arm.

Diagnosis and main eligibility criteria

Female subjects 18-50 years of age with recurrent UTI and currently on antibiotic treatment for UTI will be considered for participation in the study. Recurrent UTI is defined as occurrence of at least two UTIs during the last six months or at least three UTIs during the last 12 months with a minimum period of two weeks between episodes, unless there is a urinary culture confirming two different pathogens.

Methodology

The study will consist of a combined screening and randomization assessment, an IP first dose confirmation call and monthly follow up assessments for six months. The screening and randomization assessment will be performed as a phone call or a video-call using an e-health digital platform. Informed consent will be signed via Bank ID. The monthly follow-up assessments will be performed either as a video-call or by phone. However, physical visits to the clinic at CTC AB might be performed for subjects in Uppsala.

Subjects will be recruited from advertising (*e.g* in social media, at pharmacies etc.). The subjects will contact the study site by phone or e-mail. Brief oral information about the study will be given and pre-defined pre-screening questions will be asked. Written information will be sent to the subjects qualified for screening together with instructions regarding the e-health platform and Bank ID signing procedures.

During the screening and randomization assessment, full information about the study will be given and informed consent obtained as described in Section 13.3. Information regarding demographics, weight, height, medical history, UTI history, medication history, use of restricted products, and pregnancy will be collected and diet restrictions will be explained (see Section 10.6). Subjects will be screened for eligibility as per the pre-defined eligibility criteria (see Sections 10.4 and 10.5).

Eligible and consenting subjects will be randomized to intervention with either *Lactobacillus reuteri* or placebo. The subject will be provided with Investigational Product (IP) for three months (90 days) and niture/Leu urine test kits. Instructions to start intake of IP as soon as the IP has been picked-up will be given. The subject will be contacted by study staff 1-3 days after randomization to confirm that first IP dose has been taken

At the monthly assessments (video-call/phone-call or visit), the subject will be asked to answer pre-defined questions regarding symptoms (frequency, urgency and dysuria). If two out of three symptoms are present a UTI is confirmed. If only one symptom is present the subject will perform a niture/Leu urine test at home, preferably on urine retained in the bladder for approximately 3 hrs. If the test is positive (one of two analyses), a UTI is confirmed and the subject is recommended to contact the health care system for consultation regarding antibiotic treatment. Information on AEs, concomitant medications, use of restricted products, pregnancy, and compliance with instructions for IP intake will be collected.

An electronic diary will be used by the subject during the study period for recording of occurrence of UTI symptoms and sexual activity (frequency of intercourse, new partner, use of condom with spermicide).

The monthly follow-up assessment after six months will be the final study assessment. Number of empty sachets and any unused IP will be reported to CTC AB by the subject and documented by site staff on the IP accountability list.

Investigational Products, dosage and mode of administration

The manufacturer of the IP is Inpac in Lund AB, Lund, Sweden.

Test product:

Sachet 1 – instant drink: Xylitol, Monosodium Citrate, Cranberry Aroma, Cranberry Extract, Grape-skin Extract, Xanthan Gum, Acesulfame Potassium and Zinc Gluconate.

Sachet 2 – probiotic mix: Maltodextrin, *Lactobacillus reuteri* DSM 16666 and *Lactobacillus reuteri* DSM 17938.

Placebo:

Sachet 1 – instant drink: Xylitol, Monosodium Citrate, Cranberry Aroma, Grape-skin Extract, Xanthan Gum and Acesulfame Potassium.

Sachet 2 – maltodextrin: Maltodextrin.

Subjects will be asked to consume the IP once per day, at approximately the same time every day. Both sachets should be emptied in a glass and mixed with 200 ml of cold water.

Duration of intervention

The IP will be taken for three months (90 days).

Duration of subjects' involvement in the study

The subject will be involved in the study for six months.

Efficacy assessments

Information will be collected regarding:

- Number of confirmed UTIs
- Time from start of intervention to relapse of UTI

Safety assessments

- AE reporting

Statistical methods

Hypothesis:

H_0: Mean number of UTIs in Probiotic group = Mean number of UTIs in the placebo group

H_1: Mean number of UTIs in Probiotic group ≠ Mean number of UTIs in the placebo group

The number of subjects needed to verity that the mean number of UTIs during six months for the probiotic will be 0.75 and for placebo 1.35 with a common standard deviation of 1.15, will be 118 (1:1 randomization) using a significance level of 5% and a power of 80%. Assuming a drop-out rate of 15% a total of 140 subjects will be included.

Data on continuous variables will be presented using summary statistics. Data will be presented in terms of number (N), arithmetic mean, standard deviation (SD), minimum and maximum value.

Categorical data will be presented as counts and percentages. Individual subject data will be listed by randomization number, and where applicable by assessment time.

All statistical tests will be performed using a two-sided hypothesis with a significance level of 5%.

For the primary efficacy endpoint, the mean number of UTIs will be analysed using analysis of covariance with intervention, UTI history, age and sexual activity as independent variables. No intervention by centre interaction will be possible to apply in this analysis. The estimated intervention difference will be presented by LSMeans and a 95% confidence interval.

The Full Analysis Set (FAS) will consist of all subjects who have been randomized and received at least one dose of Intervention.

The Per Protocol Set (PPS) will consist of all subjects who have been randomized, completed the three months of intervention period and without any major protocol deviations. All deviations will be presented and discussed at the clean file meeting. If the compliance with the IP regimen should be below 80% the subject will not be included in the PPS population. The definition of compliance is by calculating the number of delivered products in relation to number of unused IP as reported by the subject.

A detailed Statistical Analysis Plan (SAP) will be approved and signed prior to clean file.

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4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or term	Explanation
AE	Adverse Event
BMI	Body Mass Index
cfu	Colony-forming units
CTA	Clinical Trial Agreement
CV	Curriculum Vitae
DMP	Data Management Plan
DSM	*Deutsche Sammlung von Mikroorganismen*
DVP	Data Validation Plan
IEC	Independent Ethics Committee
eCRF	Electronic Case Report Form
EEA	European Economic Area
FAS	Full Analysis Set
GI	Gastro-intestinal
ICF	Informed Consent Form
IP	Investigational Product
N	Number
PAC	Proanthocyanidin
PPS	Per Protocol Set
RCT	Randomized Clinical Trials
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SD	Standard Deviation
SDV	Source Data Verification
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UTI	Urinary Tract Infection
Enrolled subject	Subject who has signed the Informed Consent Form (ICF)
Screening failure	Enrolled subject not included

Included subject	Subject randomized
Withdrawn subject	Subject randomized but not completed
Completed subject	Subject completed both the intervention and the follow-up periods

5 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

The Principal Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. A medical emergency usually constitutes a Serious Adverse Event (SAE) and is to be reported as such.

In the case of a medical emergency the Investigator may contact the Medical Responsible Person at BioGaia AB:

Name	Function in the study	Telephone number and e-mail
Kerstin Nilsson	Sponsor's Project Manager	+46 73 439 7080 kn@biogaia.se

5.1 Overdose

An overdose is a dose in excess of the dose specified in this Clinical Study Protocol. Within the field of probiotics, an overdose in a healthy subject is not considered to be associated with any safety issues.

6 INVESTIGATOR(S) AND STUDY ADMINISTRATIVE STRUCTURE

This clinical study is sponsored by BioGaia AB and will be conducted by CTC Clinical Trial Consultants AB (CTC AB) in Uppsala, Sweden. Clinical conduct and management, monitoring, data management, biostatistics and medical writing will be provided by CTC AB. Key members of the Sponsor and CTC AB project teams and sub-contractors are presented below.

Sponsor

BioGaia AB
Kungsbroplan 3
SE-112 27 Stockholm, Sweden

Project Manager

Kerstin Nilsson
Phone: +46 73 439 7080
E-mail: kn@biogaia.se

Clinical conduct and management, monitoring, data management, biostatistics, and medical writing

CTC Clinical Trial Consultants AB
Dag Hammarskjölds väg 13
SE-752 37 Uppsala, Sweden

Project Manager

Anna Stendahl
Phone: +46 738 66 48 87
E-mail: anna.stendahl@ctc-ab.se

Principal Investigator

Erik Rein-Hedin
Phone: +46 70 755 20 89
E-mail: erik.rein-hedin@ctc-ab.se

Biostatistician

Fredrik Hansson, MSc
Phone: +46 721 93 55 01
E-mail: fredrik.hansson@ctc-ab.se

Manufacturer of IP, labelling

Inpac i Lund AB
Åldermansgatan 2
SE-227 64 Lund, Sweden.

Signatures required are provided in Appendix 17.1.

7 INTRODUCTION

7.1 Project background

Urinary tract infections (UTIs) are the most common bacterial infections in women, with about 50% of women experiencing at least one UTI in their lifetime [1]. Studies have shown that an UTI leads to an average of 1.2 days off work [2] and the Swedish Medical Products Agency estimates that, in Sweden, UTI cost 254 million SEK annually [3]. UTIs are usually categorized by infection site and can additionally be classified according to whether they are uncomplicated (occurring in the normal urinary tract of immunocompetent individuals, usually young healthy non-pregnant women) or complicated (occurring in individuals of all ages and sexes that are immunocompromised or have genitourinary tracts with structural or functional abnormalities, including urethral catheterisation) [4].

Most UTIs are acute uncomplicated cystitis caused by *E. coli* (86%), a gram-negative bacterium that is the major source of domestic and hospital infections [5]. Identifiers of acute uncomplicated cystitis are frequency (frequent voiding of urine), urgency (the urge to void immediately), and dysuria (pain in voiding and the absence of both fever combined with flank pain and unusual vaginal discharge) in an immunocompetent woman of childbearing age who has no comorbidities or urological abnormalities [6]. Sometimes haematuria can occur; suprapubic discomfort is less common.

From an epidemiological point of view, infections of the urinary tract and bladder are more common in women than in men [5]. About 4% of women in early adulthood experience bacteriuria and this incidence increases by about 1% to 2% per decade of age [7]. The prevalence of bacteriuria in hospitalized patients increases. In contrast, patients presenting typical UTI symptoms combined with vaginal discharge or irritation have a decreased risk of an uncomplicated UTI [8]. Most uropathogens originate in the rectal flora and enter the bladder via the urethra with an interim phase of periurethral and vaginal colonization, and sometimes they may reach the kidneys [9,10].

The main pharmacological treatments of cystitis usually involve the use of antibiotics, in particular pivmecillinam, nitrofurantoin or trimethoprim [11, 12]. Quinolones (such as ciprofloxacin and levofloxacin), fosfomycin, second-generation and third-generation cephalosporins, and b-lactam antibiotics associated with b-lactamase inhibitors [13] may also be used. Because of the formation of bacterial biofilms, unfortunately, the results of antibiotic treatment are often not satisfying. In fact, the infection is not always eradicated and bacteriuria and recurrences may persist [14, 15]. Additionally, the inadequate use of antibiotics in terms of type and duration has been a common practice, generating a continuous increase in bacterial resistance and diverse complications associated to with this indiscriminate use [16].

As alternatives or in addition to traditional treatments, there are some natural ingredients with a recognized effectiveness in counteracting the onset, persistence, or propagation of a UTI caused by *E. coli*. One example is the use of a dry extract of cranberry (Vacciunium macrocarpon), which is able to establish a natural physical-mechanical contrast against the adhesion of *E. coli* to the surface of the epithelial cells of the bladder and urinary tract [17]. Its main activity is related to the proanthocyanidin (PAC) fraction containing oligomers that compete with the adhesions located on type-P fimbriae (FimH, mannose-sensitive) used by *E. coli* to mediate its anchorage to the epithelial cells [18]. The profile of cranberry bioactives is distinct from that of other berry fruits, as they are rich in A-type PACs in contrast to the B-

type PACs present in most other fruit [19]. However, clinical studies of prevention of UTI using cranberry substances are contradictory (as reviewed by Liska, et al 2016) [20].

A recent Cochrane review included nine randomized clinical trials (RCTs) using probiotics to prevent recurrent UTIs. The authors found that there was insufficient evidence to determine if probiotics prevented UTI recurrence. The strains of lactobacteria varied between the included studies. The route of delivery and duration differed and Adverse Events (AEs) were either not reported or poorly reported. There were only six studies comparing probiotics and placebo of which only three included patients treated with antibiotics before inclusion [21, 22, 23]. None of the six studies investigated time to relapse. The authors conclude that further studies on effect of probiotics and AEs are needed

Very few studies have examined treatment effect [24, 25], of which only one is a recent pilot study demonstrating the effect of probiotics as treatment of women with UTI [24]. In this prospective pilot study, 33 premenopausal, non-pregnant women diagnosed with acute uncomplicated cystitis were enrolled and completed the treatment protocol. Women were instructed to take two doses of a substance containing lactobacteria and cranberry extract per day during the first month, and then to continue with one sachet per day until the sixtieth day. Nitrites and leukocyte esterase on urine dipstick testing were used as indicators of cystitis, with analysis performed at enrolment, after 30 and 60 days, and after one month of follow-up. Typical UTI symptoms, namely dysuria, frequent voiding of small volumes, urinary urgency, suprapubic pain, and gross haematuria were scored 0-3 and evaluated at each visit. Positive results for the presence of nitrites and leukocyte esterase were found in 14 and 20 subjects after 30 days and in nine and 14 women after 60 days, respectively ($P<0.001$). At the end of the follow-up period, positive results for nitrites and leukocyte esterase were recorded in only four and three of 24 and 19 subjects (16.7%, $P=0.103$; 15.8%, $P=0.325$, respectively), with negative results after 60 days. Typical symptoms of cystitis, specifically dysuria, frequent voiding, urgency, and suprapubic pain were significantly improved as well. No significant differences were recorded in the incidence and severity of haematuria at any visit.

7.2 Investigational Product

7.2.1 Product characteristics

Lactobacillus reuteri bacteria are active and multiply along the entire digestive tract and in addition, it has been shown that orally administered *L. reuteri* after the passage of the gastro-intestinal (GI) tract can colonise the vagina and by that modulate the vaginal microbiota in a positive manner [25, 26, 27, 28, 29, 30].

The Investigational Product (IP) consists of a combination of two *L. reuteri* strains: DSM 16666 and DSM 17938 ($5x10^8$ colony forming units [cfu]). The dose has been defined based on data from clinical trials performed with the two individual strains (see Section 7.2.2).

7.2.2 Clinical experience and mode of action

L. reuteri DSM 16666 is identical to RC-14 that together with *L. rhamnosus* GR-1 is found in the product Urex. This combination of strains has been evaluated in a number of clinical trials, using either orally or vaginally administered bacteria. The strain can colonize the vagina after oral intake and it has been shown that it could be recovered from the vagina in 9 out of 10 patients ingesting the bacteria [25]. In another study, the strain was shown to persist in the vaginal tract for more than 19 days after administration [26].

Studies with oral intake of the bacteria show that the product can modulate the vaginal microbiota in a positive manner, and, by that, counteract UTIs and bacterial vaginosis [27, 28, 29, 30]. The strain was originally isolated from the vagina of a healthy woman and has shown inhibitory effects on pathogens, probably due to the production of lactic acid and hydrogen peroxide [31, 32]. Furthermore, it prevents *in vitro* adhesion of uropathogens [33] and disrupts the biofilm produced by the vaginal pathogen *Gardnerella* [34].

L. reuteri DSM 17938 is one of the most studied probiotic bacteria and has an impressive track record. Several studies have been performed with DSM 17938 or its mother strain ATCC 55730* on adults [35, 36, 37] or infants [38, 39, 40, 41, 42, 43, 50, 51].

It was originally isolated from mother milk and has been shown to be very effective within the gastrointestinal tract. However, it does belong to a type of *L. reuteri* that often is found in the vagina. Thus, it represents a type of *L. reuteri* that is adapted to all these niches and likely will have a good persistence in the vagina.

The mode of action of the strain is thought to be: (i) survival of the GI tract passage [44]; (ii) adhesion to the vaginal mucosa similarly to the intestinal mucosa [45]; (iii) inhibiting pathogens and modulate the vaginal microbiota by production of the broad spectrum antimicrobial substance reuterin [44, 46]. Though, this strain produces hydrogen peroxide reuteri is thought to be the major antimicrobial compound; (iv) improving the mucosal integrity and by maintaining a functional barrier [47, 48] and (v) modulate the immune system to be more responsive to pathogens [49].

7.3 Study rationale

Urinary tract infections are the most common bacterial infections in women and are quite costly for society. Unrecognized or inadequately treated infections are associated with recurrent UTI which deteriorate quality of life. Additionally, there is an increasing problem with antibiotic resistance so both alternative and preventative treatments are needed.

One promising alternative to antibiotics are probiotics. As yet, there are too few randomized control studies of the efficacy of probiotics in prevention of UTIs to determine if probiotics are effective. Very few studies have examined treatment effect. To our knowledge, this will be the first RCT studying the prevention effect of the combination L. reuteri DSM 16666 and L. reuteri DSM 17938.on UTI. Moreover, it is the first study exploring time to relapse with usage of lactobacteria and will contribute to the sparse knowledge about the efficacy of lactobacteria on the number of relapses within a given time frame. Additionally, because AEs have previously been poorly studied, this study will provide further information. Thus, this RCT will provide important evidence that might support the strategic usage of probiotics in UTI, which in turn, may lead to cost reductions for society and, importantly, both reduction in antibiotic usage and antibiotic resistance development.

7.4 Risk/benefit assessment

7.4.1 Summary of risk management

Lactobacillus reuteri probiotics are safe products that have been used for treatment and prevention of many types of disorders [28, 50, 51]. The most common AE with a probable link to probiotics is the experience of flatulence that is usually restricted to the first days of intervention.

No clinical assessments will be performed in this study, thus there will be no risks associated with study procedures or devices.

Any possible AEs will be assessed in the current study.

8 STUDY OBJECTIVES AND ENDPOINTS

8.1 Primary objective

The primary objective is to evaluate the effect of oral supplementation with *Lactobacillus reuteri* on the frequency of confirmed UTI during six months from start of intervention in adult women with recurrent UTI.

8.1.1 Primary endpoint

The primary endpoint will be the mean number of confirmed UTIs during six months from start of intervention, as compared to placebo.

8.2 Secondary objectives

1. To evaluate the effect of oral supplementation with *Lactobacillus reuteri* on the frequency of confirmed UTI during the three months´ intervention period and during the three months' follow-up period, respectively.
2. To evaluate the effect of oral supplementation with *Lactobacillus reuteri* on time to first relapse of UTI.
3. To evaluate the effect of oral supplementation with *Lactobacillus reuteri* on relapse rate at three and six months after start of intervention.
4. To assess the tolerability of oral supplementation with *Lactobacillus reuteri* in adult women with recurrent UTI.

8.2.1 Secondary endpoints

- Mean number of confirmed UTIs during the three months' intervention period, as compared to placebo.
- Mean number of confirmed UTIs during the three months' follow-up period, as compared to placebo.
- Mean time from start of intervention to first relapse of UTI, as compared to placebo.
- Proportion of subjects (%) who experienced at least one confirmed UTI during three months from start of intervention, as compared to placebo.
- Proportion of subjects (%) who experienced at least one confirmed UTI during six months from start of intervention, as compared to placebo.
- Occurrence and frequency of AEs.

9 INVESTIGATIONAL PLAN

9.1 Overall study design and schedule of events

This will be a double-blinded, randomized, placebo-controlled, parallel-group study with the primary objective to evaluate the effect of oral supplementation with *Lactobacillus reuteri* on the frequency of confirmed UTI in adult women with recurrent UTIs.

The study will consist of a combined screening and randomization assessment, an IP first dose confirmation call, and monthly follow up assessments for six months. The screening and randomization assessment will be performed as a phone call or a video-call using an e-health digital platform. Informed consent will be signed via Bank ID. The monthly follow-up assessments will be performed either as a video-call or by phone. However, physical visits to the clinic at CTC AB might be performed for subjects in Uppsala.

Female subjects 18-50 years of age with recurrent UTI and currently on antibiotic treatment for UTI will be recruited from advertising (*e.g.* in social media, at pharmacies, etc.). Recurrent UTI is defined as occurrence of at least two UTIs during the last six months or at least three UTIs during the last 12 months, with a minimum period of two weeks between episodes, unless there is a urinary culture confirming two different pathogens. The subject will contact the study site by phone or e-mail. Brief oral information about the study will be given and pre-defined pre-screening questions will be asked. Written information will be sent to the subject qualified for screening together with instructions regarding the e-health platform and Bank ID signing procedures.

During the screening and randomization assessment, full information about the study will be given and informed consent obtained as described in Section 13.3. Information regarding demographics, weight, height, medical history, UTI history, medication history, use of restricted products, and pregnancy will be collected and diet restrictions will be explained (see Section 10.6). Subjects will be screened for eligibility as per the pre-defined eligibility criteria (see Sections 10.4 and 10.5).

Eligible and consenting subjects will be randomized to intervention with either *Lactobacillus reuteri* or placebo. The subjects will be provided with IP for three months (90 days) and niture/Leu urine test kits. Instructions to start intake of IP as soon as the IP has been picked-up will be given.

The subjects will be contacted by study staff 1-3 days after randomization to confirm that first IP dose has been taken.

At the monthly assessments (video-call/phone-call or visit), the subject will be asked to answer pre-defined questions regarding symptoms (frequency, urgency and dysuria). If two out of three symptoms are present a UTI is confirmed. If only one symptom is present the subject will perform a niture/Leu urine test at home, preferably on urine retained in the bladder for approximately 3 hrs. If the test is positive (one of two analyses), a UTI is confirmed and the subject is recommended to contact the health care system for consultation regarding antibiotic treatment. Information on AEs, concomitant medications, use of restricted products, pregnancy, and compliance with instructions for IP intake will be collected.

An electronic diary will be used by the subject during the study period for recording of occurrence of UTI symptoms and sexual activity (frequency of intercourse, new partner, use of condom with spermicide).

The monthly follow-up assessment after six months will be the final study assessment. Number of empty sachets and any unused IP will be reported to CTC AB by the subject and documented by site staff on the IP accountability list.

The overall study design is shown in Figure 9.1. Assessments will be performed as described in Table 9.1. Each assessment is further described in Section 12.

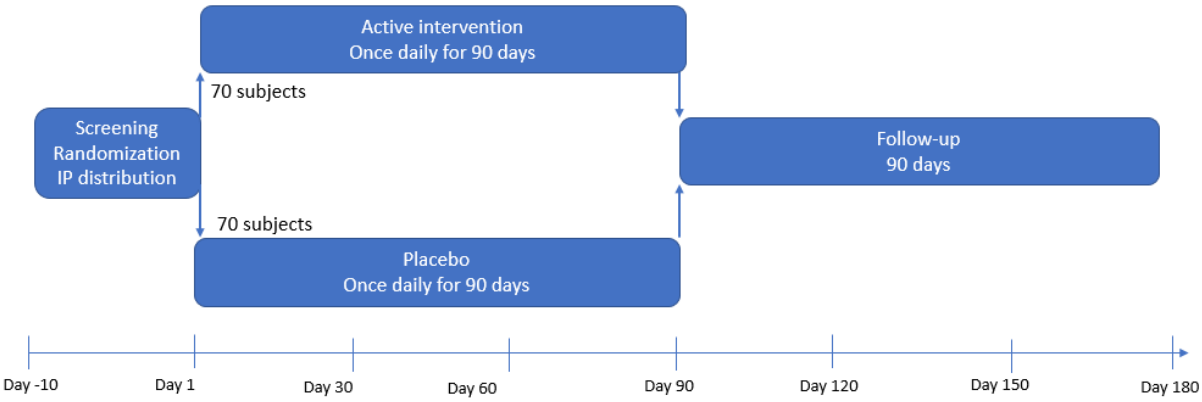

Figure 9.1 Study Flow-chart

Table 9.1 Schedule of events

	Screen/ Random.	**Intervention period**				**Follow-up**		
Assessment number	**1**	***1st dose***	**2**	**3**	**4**	**5**	**6**	**7**
Day	**-10 to 0**	**1**	**30±7**	**60±7**	**90+7**	**120±7**	**150±7**	**180±7**
Informed consent	X							
Demographics	X							
Weight and height information	X							
Medical/surgical history	X							
UTI history	X							
Medication/product history	X							
Inclusion/exclusion criteria	X							
Randomization	X							
IP distribution triggered	X							
Confirmation of IP first dose call	X[1]							
IP intake		X						
Signs of recurrent UTI			X	X	X	X	X	X
AE reporting			X	X	X	X	X	X
Pregnancy information	X		X	X	X	X	X	X
Diary recordings (ViedocMe™)[2]		X						
Concomitant medications/products[3]			X	X	X	X	X	X

[1] 1-3 days after randomization
[2] Information on sexual activity and signs of recurrent UTI.
[3] See Section 10.6 for restricted products.

9.2 Rationale for study design and dose groups

The study will be performed in female subjects aged 18-50 since this is the population expected to benefit from the intervention studied.

The study structure based on assessments using video -and phone-calls to ensure access to a broad population in a wide area although only one study site will be involved.

No clinical safety assessments are considered necessary considering the general good safety profile of probiotics.

10 STUDY POPULATION

10.1 Recruitment

Study subjects will be recruited using advertisement (*e.g* in social media, at pharmacies etc.) directed to pre-defined regions surrounding a number of IP distribution points.

10.2 Screening and enrolment log

The Investigator will keep records of all subjects screened and included. The reason for screening failure should be stated for all subjects screened but not included. The reason for withdrawal should be stated for all subjects included but not completed.

10.3 Number of subjects

A total of 140 subjects completed will be required, 70 in each intervention arm.

10.4 Inclusion criteria

For inclusion in the study, subjects must fulfil the following criteria:

1. Willing and able to give informed consent for participation in the study.
2. Woman aged 18-50 years old, both inclusive, at screening.
3. Body Mass Index (BMI) ≥18 at screening.
4. Recurrent UTI, otherwise healthy. Recurrent UTI is defined as occurrence of at least two UTIs during the last six months or at least three UTIs during the last 12 months with a minimum period of two weeks between episodes, unless there is urinary culture confirming two different pathogens.
5. Currently on antibiotic treatment for UTI, at the time of screening 1.
6. Able and willing to comply with the restrictions defined for the study period (see Section 10.6).

[1] The intake of IP must be started during or within five days from stop of current antibiotic treatment for UTI.

7. Access to Bank ID and ability to use the e-health platform (i.e. Internet access).
8. Ability to understand and comply with the requirements of the study, as judged by the Investigator.

10.5 Exclusion criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled:

1. Postmenopausal (defined as 12 months of amenorrhoea).
2. Pregnant or breastfeeding.
3. Planning to become pregnant during the study.
4. Irregular menstruations combined with perimenopausal symptoms.
5. Known hypersensitivity or allergy to any of the components of the test product, or to the comparator (placebo).
6. Currently on long-term antibiotic treatment.
7. Use of spermicide contraceptives including spermicidal condom within three days prior to first IP dose.
8. History of complicated cystitis, urgency incontinence, recent pyelonephritis, urological and/or gynaecologist abnormalities, as judged by the Investigator.
9. Unstable bowel disorder such as chronic or recurring diarrhoea, Crohn's disease, ulcerative colitis.
10. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.
11. Participation in any other clinical interventional study within 30 days before treatment.

10.6 Restrictions during the study

10.6.1 General restrictions

Unprotected sexual contact with new male partner will not be allowed during the study to avoid chlamydia symptoms being similar to UTI symptoms.

The subject must be willing and able to comply with the instructions for storage of the IP given (see Section 11.3).

10.6.2 Prior and concomitant therapy

No probiotic food supplements, probiotic foods or fermented food other than the test product, including vaginal products containing *Lactobacillus* (including probiotic tampons) or affecting the pH, are allowed from screening and throughout the study period.

Neither is any food or food supplements containing cranberries allowed from screening and throughout the study period. Subjects should continue with the same general diet and lifestyle, except probiotic and cranberry consumption.

Use of spermicide contraceptives including spermicidal condom will not be allowed during the study, up to Day 180.

Use of oral or vaginal fungicides will not be allowed during the study, up to Day 180. If typical vaginal candida symptoms appear (itching and typical thick, clumpy, white vaginal discharge) sporadic usage of oral fluconazole 150 mg as a single dose may be allowed and should be noted.

Subjects must not participate in any other clinical interventional study during the study period.

Other medications considered necessary for the subject's safety and well-being, may be used during the study period. In case of antibiotic use, the type and duration should be recorded. Any use of fluconazole, prohibited medication, therapies or products will require evaluation by the Investigator to determine whether or not the subject should continue in the study. This may be done in consultation with the Sponsor.

10.7 Criteria for subject withdrawal

Subjects are free to discontinue their participation in the study at any time and for whatever reason without affecting their right to an appropriate follow-up assessment or their future care. If possible, the reason for withdrawal of consent should be documented.

Subjects may be discontinued from the study at any time at the discretion of the Investigator for any of the following reasons:

- Severe non-compliance to Study Protocol procedures, as judged by the Investigator and/or Sponsor.
- Subject is lost to follow-up.
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or Sponsor

10.7.1 Procedures for discontinuation of a subject from the study

A subject who prematurely discontinues participation in the study will always be asked about the reason(s) for discontinuation and the presence of any AEs. If possible, they will be contacted by the Investigator and assessed according to the procedures scheduled for the follow-up assessment, as far as possible. Any ongoing AEs will be followed as described in Section 12.5.6.

All data collected until discontinuation will be used in the study.

10.7.2 Subject replacement

Withdrawn subjects will not be replaced.

10.8 Randomization

Subjects will be randomized to treatment with active test product or placebo (1:1). A computer-generated randomization list will be created by CTC AB. The randomization will be performed using block randomization within each IP distribution site (these sites will be identified by the Sponsor prior to creating the randomization list). Each distribution site will receive pre-packed boxes with either the test product or placebo, each box marked with a randomization number dedicated to the subject at randomization. The clinic will have a sub-part of the randomization list which contains information about randomization number and distribution site.

10.9 Blinding

This is a double-blinded study. All study staff, including the Principal Investigator and the persons performing the subject assessments using the video link or phone calls, will be blinded during the study. Sealed individual treatment code envelopes will be kept at the clinic to be able to break the code if any emergency occurs, as judged by the Investigator.

10.10 Emergency decoding of blinded intervention during the study

The intervention code may only be broken by the study medical staff in case of emergency when knowledge of the intervention received is necessary for the proper medical management of the subject. The code breaking procedure should be carefully documented in the medical records.

11 INTERVENTIONS

11.1 Identity of Investigational Products

The manufacturer of the IP is Inpac in Lund AB, Lund, Sweden. The two IPs (test product and placebo) each contains two sachets.

11.1.1 Test product

Sachet 1 – instant drink: Xylitol, Monosodium Citrate, Cranberry Aroma, Cranberry Extract, Grape-skin Extract, Xanthan Gum, Acesulfame Potassium and Zinc Gluconate.

Sachet 2 – probiotic mix: Maltodextrin, *Lactobacillus reuteri* DSM 16666 and *Lactobacillus reuteri* DSM 17938.

11.1.2 Placebo

Sachet 1 – instant drink: Xylitol, Monosodium Citrate, Cranberry Aroma, Grape-skin Extract, Xanthan Gum and Acesulfame Potassium.

Sachet 2 – maltodextrin: Maltodextrin.

11.2 Packaging, labelling

Labels will comply with applicable Good Manufacturing Practice (GMP) requirements (EudraLex *VOLUME 4, Good manufacturing practices, ANNEX 13, Manufacture of investigational medicinal, products, February 2010, section 26*).

11.3 Conditions for storage

The IP should be stored at +2 to +8°C. Temperature control will be performed once daily at the distribution sites (business days only). When the IP has been distributed to the subject, it should be refrigerated as soon as possible and kept in the refrigerator until use. A thermal bag will be provided to each subject. The product can be stored in the thermal bag for a maximum of 12 hrs.

11.4 Dispensing and accountability

The IP will be picked-up by the subject at local IP distribution sites. The subject will receive 90 packages of IP. The IP can be picked-up at several occasions, if preferred by the subject. At the last assessment of the intervention period (Day 90), the subject will inform CTC AB about the number of empty sachets and any unused IP for accountability and check of intervention compliance.

Each IP distribution site will maintain an IP accountability list detailing the dates and quantities of the IP allocated to each subject. Clinical staff will record any unused IP on the IP accountability list, based on information given by the subject.

11.5 Administration of Investigational Product

The IP will be self-administered by the subject. Subjects will be asked to consume the IP (two sachets) once per day, at approximately the same time every day, for 90 days. Both sachets should be emptied in a glass and mixed with 200 ml of cold water.

The intake of IP must be started during or within five days from stop of current antibiotic treatment for UTI.

11.6 Continuation of intervention with Investigational Product

There will be no intervention with the current IP available for the study subjects after end of study participation.

11.7 Compliance with the intervention

During the monthly assessments (video-calls/phone-calls or visits), the subject will be asked if the IP has been stored and taken according to instructions and any deviations will be recorded.

11.8 Return and destruction of Investigational Product

Any unused IP will be destroyed by the subject and reported to the clinical staff at CTC AB after completion of the intervention period. The Monitor will check the IP accountability list to verify that all unused IP is adequately documented.

12 STUDY ASSESSMENTS

The study assessments are described in the sections below and the timing of these assessments are detailed in the schedule of events (Table 9.1, Section 9.1).

12.1 Recording of data

The Principal Investigator at the study site will provide the Sponsor with all data produced during the study from the scheduled study assessments. He/she ensures the accuracy, completeness, legibility, and timeliness of the data reported to Sponsor in the electronic Case Report Form (eCRF) and in all required reports.

12.2 Technical tools used

12.2.1 E-Health digital platform

The system for electronic signature of the Informed Consent Form (ICF) using Bank-ID for identity authentication will be provided by *Assently*. The system (web interface) will be accessed via Internet. Signed ICF´s will be stored in *Assently* encrypted, secured server which is located in Sweden. A link to the signed ICF will be sent via email to the subject and responsible clinical staff. The signed ICF will be available for monitoring and auditing purposes, when required.

The e-health platform for video-call (the system) will be provided by *VisibaCare*. The system (web interface) will be accessed via Internet and the subject will log-in to the system using Bank-ID for identity authentication. All communications during video calls are secured, encrypted and handled in accordance with the Data Protection law. The information will be stored in *VisibaCare* encrypted, secured server which is located in Sweden.

A telephone call could also be used instead of video-call. The clinical personnel will ask the subject to identify herself by her personal identification number.

12.2.2 ViedocMe electronic diary

An electronic diary will be used by the subject during the study period for recording of occurrence of UTI symptoms and sexual activity (frequency of intercourse, new partner, use of condom with spermicide).

12.3 Demographics and other baseline characteristics

12.3.1 Informed consent

Informed consent must be obtained before any screening procedures are initiated. The informed consent will be signed via Bank ID. The informed consent procedure is further described in Section 13.3.

12.3.2 Demographic information

The following demographic data will be recorded: age, and ethnic origin.

12.3.3 Weight and height

Weight and height will be recorded based on information given by the subject. Body Mass Index will be calculated from the height and weight recorded and rounded to the nearest whole number.

12.3.4 Medical/surgical history

Medical/surgical history will be obtained during the screening interview to confirm eligibility.

12.3.5 Urinary tract infection history

Information regarding previous UTIs (diagnosis, prescriptions, etc.) during the last 12 months will be collected to confirm eligibility.

12.4 Efficacy assessments

12.4.1 Signs of recurrent UTI

At the monthly assessments, the subjects will be asked to answer pre-defined questions regarding UTI symptoms: frequency (frequent voiding of urine), urgency (the urge to void immediately), and dysuria (pain in voiding and the absence of both fever combined with flank pain and unusual vaginal discharge). If two out of three symptoms are present a UTI is confirmed. If only one symptom is present the subject will perform a niture/Leu urine test at home, preferably on urine retained in the bladder for approximately 3 hrs. If the test is positive (one of two analyses), a UTI is confirmed and the subject is recommended to contact the health care system for consultation regarding antibiotic treatment.

12.5 Adverse Events

In this study, the IP is a food supplement and not an investigational medicinal product. However, the procedures for monitoring, collecting and reporting of AEs will be the same as for an investigational medicinal product, as far as possible.

12.5.1 Event definitions

12.5.1.1 Adverse Event

An Adverse Event is any untoward medical occurrence in a clinical study subject administered an IP and which does not necessarily have a causal relationship with this intervention. An AE can therefore be any unfavourable and unintended sign (including a significant abnormal laboratory value), symptom, or disease temporally associated with the use of an IP, regardless of whether it is considered related to the IP.

12.5.1.2 Serious Adverse Event

An SAE is any AE that:

- results in death
- is life-threatening (this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death had it been more severe)
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is medically important (this refers to an event that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent any of the SAEs defined above)

Examples of medically important events are intensive treatment in an emergency room for allergic bronchospasm or blood dyscrasias, convulsions that do not result in hospitalisation, development of drug dependency, and drug abuse.

Planned hospitalisations or surgical interventions for a condition that existed before the subject signed the ICF and that did not change in intensity are not SAEs.

If there is any doubt as to whether an AE meets the definition of an SAE, a conservative viewpoint must be taken, and the AE must be reported as an SAE.

12.5.1.3 Serious Adverse Reaction

The term Serious Adverse Reaction (SAR) is to be used whenever either the Investigator or Sponsor or designee assessed the SAE as possibly or probably related to the IP.

12.5.1.4 Suspected Unexpected Serious Adverse Reaction

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is any SAR whose nature or intensity is not consistent with the current product information.

12.5.2 Adverse Event assessment definitions

12.5.2.1 Assessment of severity/intensity

The Investigator must assess the *severity/intensity* of an AE using the following definitions, and record it on the *Adverse Event Form* in the eCRF:

Mild	The AE does not interfere in a significant manner with the subject's normal functioning level. It may be an annoyance.
Moderate	The AE produces some impairment of function but not hazardous to health. It is uncomfortable and/or an embarrassment.
Severe	The AE produces significant impairment of functioning or incapacitation and/or it is a hazard to the subject.

12.5.2.2 Assessment of causal relationship

The Investigator must assess the *causal relationship* between an AE and the IP using the definitions below and record it on the *Adverse Event Form* in the eCRF as well as on the *Serious Adverse Event Report Form*, if applicable:

- *Probable* – the AE has a strong temporal relationship to the IP or recurs on re-challenge, and another aetiology is unlikely or significantly less likely.
- *Possible* – the AE has a suggestive temporal relationship to the IP, and an alternative aetiology is equally or less likely.
- *Not related* – the AE has no temporal relationship to the IP or is due to underlying/concurrent illness or effect of another drug (that is, there is no causal relationship between the IP and the AE).

An AE is considered causally related to the use of the IP when the causality assessment is *probable* or *possible*. For an SAE, a causality assessment is also made by the Sponsor.

12.5.2.3 Assessment of Outcome

The Investigator must assess the *outcome* of an AE using the definitions below and record it on the *Adverse Event Form* in the eCRF:

- *Recovered* – the subject has recovered completely, and no symptoms remain.
- *Recovering* – the subject's condition is improving, but symptoms still remain.
- *Recovered with sequelae* – the subject has recovered, but some symptoms remain (for example, the subject had a stroke and is functioning normally, but has some motor impairment).
- *Not recovered* – the subject's condition has not improved and the symptoms are unchanged (for example, an atrial fibrillation has become chronic).
- *Death*

12.5.3 Collecting Adverse Events

AEs identified using any of the following methods will be recorded:

- AEs spontaneously reported by the subject
- AEs observed by the Investigator or medical personnel
- AEs elicited based on non-leading questions from the Investigator or medical personnel

Collection of AEs starts after the subject signs the ICF and continues until the last follow-up assessment. At the last follow-up assessment, information on new AEs or SAEs, if any, and stop dates for AEs recorded and on-going during the dosing period must be recorded.

12.5.4 Recording Adverse Events

Adverse Events must be recorded on an *Adverse Event Form* in the eCRF. The Investigator must provide information on the AE, preferably with a diagnosis or at least with signs and symptoms; start and stop dates, start and stop time; intensity; causal relationship to IP; action taken, and outcome.

If the AE is serious, this must be indicated in the eCRF. Furthermore, the Investigator must fill out the *Serious Adverse Event Report Form* and report the SAE to the Sponsor as described in Section 12.5.5.

AEs, including out-of-range clinically significant clinical safety laboratory values, must be recorded individually, except when considered manifestations of the same medical condition or disease state; in such cases, they must be recorded under a single diagnosis.

If the severity/intensity of an AE increases a new *Adverse Event Form* must be completed in the eCRF.

12.5.5 Reporting Serious Adverse Events

The Investigator will be made aware of an SAE during the scheduled follow-up assessment (video-call/phone calls or visit) or in case the subject contacts the site between scheduled visits. From start of IP administration, all SAE must be reported to the Sponsor within 24 hrs of when the Investigator became aware of the SAE. The SAE should be mailed to the Sponsor SAE mailbox at saeclinicaltrials@biogaia.se.

The same information must also be sent to CTC AB SAE email inbox: sae@ctc-ab.se

To report SAEs, the *Serious Adverse Event Report Form* for clinical studies provided must be used. The first report should contain as much information as possible, and if more information about the subject's condition becomes available a follow-up report must be submitted with the additional information using the same procedure as for the initial report.

The Sponsor or a delegate will assume responsibility for reporting SAEs to applicable IEC in accordance with local regulations.

The Sponsor will assess expectedness and inform all the Investigators about any SUSARs.

12.5.6 Treatment and follow-up of Adverse Events

Subjects with AEs that occur during the study must be treated according to daily clinical practice at the discretion of the Investigator.

AEs must be followed up until resolution or the final study assessment, whichever comes first.

It is the responsibility of the Investigator to follow up on all SAEs until the subject has recovered, stabilized, or recovered with sequelae, and to report to the Sponsor all relevant new information using the same procedures and timelines as those for the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultation.

SAEs spontaneously reported by a subject to the Investigator within 30 days after the last follow-up assessment must be handled in the same manner as SAEs occurring during the study. These SAEs will be reported to the Sponsor.

12.6 Appropriateness of measurements

The screening and randomization assessment will be performed as a phone call or a video-call using an e-health digital platform (see Section 12.2.1) and the follow-up assessments will be performed either as video-calls or by phone. In case a visit to the clinic is performed for subjects in Uppsala, the same information will be collected as planned for the video/phone-calls, thus no clinical assessments will be performed.

13 ETHICAL AND REGULATORY REQUIREMENTS

13.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (see Appendix 17.2) and applicable local regulatory requirements.

A link to the Declaration of Helsinki is included in Appendix 17.2.

13.2 Ethics review

CTC AB Uppsala, on behalf of the Sponsor, is responsible for submission of the Clinical Study Protocol, the subject information and ICF, any other written information to be provided to the subjects and any advertisements used for recruitment of subjects to the applicable IEC for approval. Approval must be obtained in writing from IEC before the first subject can be recruited.

13.3 Subject information and consent

It is the responsibility of the Investigator or an authorised associate to give each potential study subject adequate verbal and written information before any study specific assessments are performed. Brief oral information about the study will be given during the initial pre-screening call. Written information will be sent to the subjects qualified for screening. During the screening and randomization assessment, full oral information about the study will be given. The informed consent must be signed by the subject and by the person who conducted

the informed consent discussion before any study-related information is recorded or procedures performed (*e.g*. randomization, IP distribution) using Bank ID.

The information will include the objectives and the procedures of the study as well as any risks or inconvenience involved. It will be emphasised that participation in the study is voluntary and that the subject may withdraw from participation at any time and for any reason, without any prejudice. All subjects will be given the opportunity to ask questions about the study and will be given sufficient time to consider participation before signing the ICF.

The subject information including the signed ICF will be made available to the subjects via the e-health platform.

Documentation of the discussion and the date of informed consent must be recorded in the source documentation and in the eCRF. The subject information sheet and the signed ICF should be filed by the Investigator for possible future audits and/or inspections.

The final approved version of the subject information and ICF must not be changed without approval from the Sponsor and the applicable IEC.

13.4 Subject data protection

The ICF includes information that data will be recorded, collected and processed and may be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the European Union Data Protection Directive (95/46/EC [Regulation 2016/679 from 2018-05-25]), the data will not identify any persons taking part in the study.

The potential study subject should be informed that by signing the ICF she approves that authorized representatives from Sponsor, CTC AB, and the concerned IEC have direct access to her medical records for verification of clinical study procedures. An authorization from the clinic for direct access to medical records must be available.

The subject has the right to request access to her personal data and the right to request rectification of any data that is not correct and/or complete.

The Investigator must file a Subject Identification List which includes sufficient information to link records, *i.e*. the eCRF and clinical records. This list should be preserved for possible future inspections/audits but should not be made available to the Sponsor except for monitoring or auditing purposes.

13.5 Changes to the approved Clinical Study Protocol

Any proposed change to the approved Final Clinical Study Protocol (including appendices) will be documented in a written and numbered Clinical Protocol Amendment. All amendments including substantial changes to the protocol must be approved by the appropriate IEC before implementation according to applicable regulations.

13.6 Audits and inspections

Authorized representatives from the Sponsor or an IEC may perform audits or inspections at the research clinic, including Source Data Verification (SDV). The purpose of an audit or inspection is to systematically and independently examine all study-related activities and

documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol and any applicable regulatory requirements. The Principal Investigator will contact the Sponsor immediately if contacted by the IEC about an inspection at the site.

13.7 Insurance

Subjects will be covered under BioGaia AB liability insurance policy. The certificate of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating subjects are also protected in accordance with national regulations, as applicable. CTC AB has a company insurance covering services performed by CTC AB.

14 STUDY MANAGEMENT

14.1 Training of study site personnel

Before enrolment of the first study subject a Sponsor representative or delegate will perform a Study Initiation Visit at the research site. The requirements of the Clinical Study Protocol and related documents will be reviewed and discussed and the investigational staff will be trained in any study specific procedures and system(s) utilised.

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, and have a detailed knowledge of and training in the procedures that are to be executed by them. Any new information of relevance to the performance of this study must be forwarded to the staff involved in a timely manner.

The Principal Investigator will keep a list of all personnel involved in the study together with their function and study related duties delegated. A Curriculum Vitae (CV) will be available for all staff delegated study-specific duties.

14.2 Clinical Monitoring

The study site will be periodically visited by a Monitor from an independent group at CTC AB at times agreed on by the Investigator and the Monitor. At the time of each monitoring visit, the function of the Monitor is to:

- provide information and support to the investigational team.
- confirm that facilities and resources remain acceptable.
- confirm that the investigational team is adhering to the Clinical Study Protocol, applicable Standard Operating Procedure (SOPs), guidelines, manuals and regulatory requirements.
- verify that data are being accurately and timely recorded in the eCRFs.
- verify that data in the eCRF are consistent with the clinical records (SDV).
- verify that the correct informed consent procedure has been adhered to for participating subjects.

- verify that AEs are recorded and reported in a timely manner and according to the Clinical Study Protocol.

When the study has been completed and all queries have been resolved and the database has been locked, the Monitor will perform a close-out visit.

14.3 Source data document

A separate source data document will be generated before start of enrolment, specifying the location of the source of derived information appearing in the eCRF. This document must be signed by the Principal Investigator and the Monitor to confirm agreement before start of recruitment.

The Principal Investigator should guarantee access to source documents to the Monitor and the IEC, if required.

14.4 Study agreements

The Principal Investigator must comply with all the terms, conditions, and obligations of the Clinical Trial Agreement (CTA) for this study.

Agreements between Sponsor and CTC AB must be in place before any study-related procedures can take place, or subjects be enrolled.

14.5 Study time table and end of study

The end of the clinical part of the study is defined as the last follow-up assessment of the last subject participating in the study.

The study is expected to start in Quarter 4, 2017 and to be completed by Quarter 4, 2018.

14.6 Discontinuation of the study

The Sponsor reserves the right to discontinue the study at any time, but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the Investigator must inform all participating subjects and perform relevant assessments, preferably according to the scheme for the final assessments. All delivered and unused study products and other study materials must be returned and all eCRFs completed as far as possible.

14.7 Reporting and publication

14.7.1 Clinical Study Report

A Clinical Study Report describing the conduct of the study, the statistical analysis performed and the results obtained, will be prepared by CTC AB. The report will be reviewed and approved by, as a minimum, the Principal Investigator, the Statistician and the Sponsor.

The final Clinical Study Report must be submitted to the IEC within 12 months after completion of the study.

14.7.2 Confidentiality and ownership of study data

Any confidential information relating to the IP or the study, including any data and results from the study, will be the exclusive property of the Sponsor. The Investigator and any other persons involved in the study will protect the confidentiality of this proprietary information belonging to the Sponsor.

14.7.3 Publication

The results from this study will be submitted for publication at the discretion of the Sponsor.

14.8 Archiving

The Principal Investigator is responsible for maintaining essential documents, for 10 years after finalization of the Clinical Study Report. This includes any original source documents related to the study, the Subject Identification List (providing the sole link between named subject source records and anonymous CRF data), the original signed ICFs and detailed records of disposition of IP.

It is the responsibility of the Sponsor to inform the Principal Investigator/institution as to when these documents no longer need to be retained.

The Sponsor will archive the Trial Master File (TMF) and applicable regulatory requirements.

15 DATA MANAGEMENT

15.1 Case Report Forms

15.1.1 Viedoc™

Data will be collected in eCRFs (Viedoc™) specifically designed for this study. The Investigator or an authorized person will record subject data in the eCRF in a precise and accurate manner. Abbreviations should not be used. The Investigator is responsible for the data entered and sign off the eCRF at each visit and at the end of the study. The data should be recorded as soon as they are generated. Only persons authorized by the Investigator are allowed to make entries to the eCRF.

An electronic diary will be used by the subject during the study period for recording of occurrence of UTI symptoms and sexual activity.

15.1.2 ViedocMe™

An electronic diary will be used by the subject during the study period for recording of occurrence of UTI symptoms and sexual activity (frequency of intercourse, new partner, use of condom with spermicide). A separate invitation will be sent to each included subject directly from the Viedoc™ system. The CTC AB clinic will save the sent-out invitation for security purpose. The invitation will consist of a URL, Username and Passcode. The subject will be by the system advised to change the pass code at first login.

15.2 Data management plan and database design

Detailed information on data management will be described in a study-specific Data Management Plan (DMP).

The person entering data into the database is not allowed to attempt any personal interpretation or to make any decisions on the data other than self-evident corrections as listed in the study-specific Data Entry Instructions or Data Handling Report.

Data validation/data cleaning procedures are designed to assure validity and accuracy of clinical data. These procedures consist of manual reviewing during data entry and computerized edit checks and queries for identifying data values that are outside the allowed range, protocol violations, incomplete or inconsistent. A Data Validation Plan (DVP) will specify the checks that are to be performed on subject data for the study. All study-specific and standard data validation programming will be tested in a separate testing environment prior to use on production data.

Queries in Viedoc™ can be raised by Monitors, Data Managers, medical coding team, and medical safety representatives. Queries raised may be related to missing or questionable data found during data validation, SDV, review, reconciliation of SAEs, and/or coding of study data. All queries should be written and answered in English.

The Investigator or designated site personnel will provide answers to all queries by giving a clear explanation to the question asked. The site Monitor is responsible for approving the resolved query. In case the answer to a query was not satisfactory the query should be rejected and re-phrased if needed. Rejected queries will be re-issued automatically and listed as unresolved.

15.2.1 Database lock

When all data have been entered, discrepancies solved and all reconciliation with the SAE database is complete, the database will be locked and the data will be analysed.

16 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The principal features of the statistical analysis to be performed are described in this section. A more technical and detailed elaboration of the principal features will be presented in a separate Statistical Analysis Plan (SAP).

16.1 General

Data on continuous variables will be presented using summary statistics. Data will be presented in terms of number (N), arithmetic mean, standard deviation (SD), minimum and maximum value.

Categorical data will be presented as counts and percentages. Individual subject data will be listed by randomization number, and where applicable by assessment time.

All statistical tests will be performed using a two-sided hypothesis with a significance level of 5%.

16.2 Determination of sample size

Hypothesis:

H_0: Mean number of UTIs in Probiotic group = Mean number of UTIs in the placebo group

H_1: Mean number of UTIs in Probiotic group ≠ Mean number of UTIs in the placebo group

The number of subjects needed to verity that the mean number of UTIs during six months for the probiotic will be 0.75 and for placebo 1.35 with a common standard deviation of 1.15, will be 118 (1:1 randomization) using a significance level of 5% and a power of 80%. Assuming a drop-out rate of 15% a total of 140 subjects will be included.

16.3 Analysis data sets

16.3.1 Full Analysis Set

The Full Analysis Set (FAS) will consist of all subjects who have been randomized and received at least one dose of intervention.

16.3.2 Per Protocol Set

The Per Protocol Set (PPS) will consist of all subjects who have been randomized, completed the three months of intervention period and without any major protocol deviations. All deviations will be presented and discussed at the clean file meeting. If the compliance with the IP regimen should be below 80% the subject will not be included in the PPS population. The definition of compliance is by calculating the number of delivered products in relation to the number of unused IP as reported by the subject.

16.4 Description of study population

16.4.1 Demographics and baseline data

Summary statistics of demographics and other baseline characteristics will be presented by intervention group.

16.4.2 Medical history and concomitant medications

Medical/surgical history and prior/concomitant medications/products will be presented by descriptive statistics and listings by intervention.

16.4.3 UTI history

The number of previous UTIs (prior to screening) will be presented using frequency tables.

16.4.4 Compliance with the intervention

Compliance with the intervention will be calculated as number of delivered products divided by number of reported unused products and presented using summary statistics.

16.5 Analysis of primary endpoint

The primary endpoint will be the mean number of confirmed UTIs during six months from start of intervention, as compared to placebo.

The mean number of UTIs per intervention group will be estimated based on the total number of UTIs by subject.

The mean number of UTIs will be analysed using analysis of covariance with intervention, UTI history, age, and sexual activity as independent variables. No intervention by centre interaction will be possible to apply in this analysis. The estimated intervention difference will be presented by LSMeans and a 95% confidence interval.

16.6 Analysis of secondary efficacy endpoints

16.6.1 Number of confirmed UTIs

The number of confirmed UTIs for both the:

- Mean number of confirmed UTIs during the three months' intervention period, as compared to placebo.

and

- Mean number of confirmed UTIs during the three months' follow-up period, as compared to placebo.

will be analysed using the same methods as for the primary endpoint.

16.6.2 Time to first relapse of UTI

Time to first relapse of UTI will be defined as the time from delivery of intervention (*i.e.* start of intervention) to date of first confirmed UTI ((Confirm date – Intervention date) + 1). Subjects with no UTI will be censored at last known date within the study period.

The time to first relapse will be analysed using Cox regression analysis and presented by Kaplan-Meier graphs.

16.6.3 Relapse rate

The relapse rate for both:

- Proportion of subjects (%) who experienced at least one confirmed UTI during three months from start of intervention, as compared to placebo.

and

- Proportion of subjects (%) who experienced at least one confirmed UTI during six months from start of intervention, as compared to placebo.

will be analysed using Chi-square test without continuity correction and presented using frequency tables.

16.7 Analysis safety endpoints

16.7.1 Adverse Events

Adverse Events and SAEs will be recorded from start of IP intervention. Medical events occurring between screening and start of IP intervention will be reported separately.

16.8 Statistical/analytical issues

16.8.1 Adjustments for covariates

History of UTIs, age, and sexual activity will be used as covariates in the analysis of mean number of UTIs.

16.8.2 Handling of dropouts or missing data

No imputation of missing data will be performed.

16.8.3 Multi-centre studies

No adjustment for centre will be applied, since this will not be considered as a multi-centre study.

16.8.4 Multiple comparison/multiplicity

All statistical significant findings need to be interpreted from a medical perspective and judged as clinical relevant or not and therefore no adjustment for multiplicity will be performed.

16.8.5 Examination of subgroups

To be addressed in the SAP.

16.8.6 Interim analyses

No interim analysis will be performed.

17 APPENDICES

17.1 Signature page

"I agree to the terms of this Clinical Study Protocol."

Sponsor signatories

Kerstin Nilsson		
Name	*Signature*	*Date*

Principal Investigator

Erik Rein-Hedin, MD		
Name	*Signature*	*Date*

17.2 Declaration of Helsinki

http://www.up.ac.za/media/shared/Legacy/sitefiles/file/45/2875/declarationofhelsinki_fortaleza_brazil2013.pdf

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