MULTICENTRIC PILOT STUDY TO COMPARE THE EFFECTIVENESS OF VAGINAL CAPSULES WITH BORIC ACID AND *L. GASSERI* AND *L. RHAMNOSUS* VERSUS ANOTHER DRUG WITH THE SAME ROUTE OF ADMINISTRATION IN PATIENTS WITH VULVOVAGINITIS DUE TO BACTERIAL OR CANDIDAL AETIOLOGY

Protocol code: DOBO-01-16

CLINICAL TRIAL PROTOCOL

(Version 1.5)

Principal Investigator and Coordinator: [redacted]

Collaborating Investigators: [redacted]

Type of Document: Clinical Trial Protocol

Development Phase: Postmarketing

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CONFIDENTIAL

PROTOCOL SIGNATURE PAGE

DOBO-01-16 PILOT STUDY PROTOCOL

I have read this protocol and agree to conduct this clinical trial in accordance with all the protocol's stipulations, current legislation and the Helsinki Declaration.

Investigator:

_____	_____	_____
Name	Signature	Date

Co-investigator:

_____	_____	_____
Name	Signature	Date

EMERGENCY TELEPHONE NUMBERS

In the event of an emergency, quickly call the doctor responsible and/or the person responsible for monitoring the trial.

INVESTIGATOR RESPONSIBLE

TRIAL MONITORING			
Person responsible for monitoring the trial	Telephone number during office hours	Telephone number after office hours	Fax number

1 SUMMARY

1.1 Title

Multicentric pilot study to compare the effectiveness of vaginal capsules with boric acid and *L. gasseri and L. rhamnosus* versus another drug with the same route of administration in patients with vulvovaginitis due to bacterial or candidal aetiology.

1.2 Protocol code

EUDRACT Code: 2016-000371-24

Sponsor Code: DOBO-01-16

1.3 Sponsor

Laboratorios Ordesa, S.L.
Carretera del Prat, 9-11;
08830, Sant Boi de Llobregat, Barcelona.

Medical Management:

[redacted]
Gastroenterology specialist doctor

Medical Management Technician:

[redacted]
Dietician-nutritionist

1.4 Principal investigator and study coordinator

Dr [redacted]
Gynaecology Department

Seville

1.5 Collaborating investigators

Dr [redacted]
Gynaecology Department
Seville.

Dr [redacted]
Gynaecology Department
Zaragoza.

Dr [redacted]
Gynaecology Department
Zaragoza.

1.6 Centres where the study is scheduled to be performed

National gynaecology and emergency clinics:

1. **Seville**

2. **Zaragoza**

1.7 Ethical Review Committees (ERC)

All the study materials have been reviewed and approved by:

- ERC of the Virgen Macarena-Virgen del Rocío University Hospitals (Seville) (rERC)
- Aragon ERC *(CEICA)*

The **Biomedical Research Ethics Coordinator Committee of Andalucía (CCEIBA)** will be the trial coordinator in Andalusia.

1.8 Study healthcare product and control treatment

- **Study healthcare product:** boric acid 150 mg, *Lactobacillus gasseri, Lactobacillus rhamnosus* and prebiotics.
- **Pharmaceutical form:** Capsule
- **Route of administration:** Vaginal
- **Therapeutic group:** D08AD. Antiseptics and disinfectants - Products with boric acid**.**

- **Control treatment (I):** Clotrimazole 100 mg (Gine-Canesten®).
  - **Pharmaceutical form:** Tablet
  - **Route of administration:** Vaginal
  - **Therapeutic group:** D01AC. Antifungal agents for topical dermatological use - Imidazole and triazole derivatives.
- **Control treatment (II):** Clindamycin 100 mg (Dalacin®)
  - **Pharmaceutical Form:** Suppository
  - **Route of administration:** Vaginal
  - **Therapeutic Group:** J01FF. Lincosamide macrolides and Streptogramins - Lincosamides.

1.9 Clinical trial phase

Postmarketing.

1.10 Objectives

Main:

To assess the effectiveness of a vaginal application formula with boric acid and probiotics for the treatment of symptomatic episodes of vulvovaginitis (VV) against a control pharmacological treatment (according to the VV aetiology).

Secondary:

- To determine the incidence of recurrence/relapse at 3 months.
- To assess the level of post-treatment vaginal flora restoration.
- To characterise the different symptomatic and microbiological profiles of vulvovaginitis.
- To assess the tolerance and safety of the product.

1.11 Study design

Multicentric, Open, Prospective, Drug-controlled Randomised Clinical Trial.

1.12 Disease under study

Infectious vulvovaginitis (including vulvovaginal candidiasis and bacterial vaginosis).

1.13 Type of study

Clinical trial with healthcare products and pharmaceutical specialities. Pilot study.

1.14 Study population

Women over 18 years of age who consult the gynaecologist due to suspicion of infectious vulvovaginitis.

1.15 Monitoring

[redacted] will be responsible for the logistics and monitoring of the study.

1.16 Treatment duration

Healthcare product subject to study: 7 days
Control drug (I): 6 days
Control drug (II): 3 days

1.17 Expected clinical trial duration

The planned duration for the recruitment of patients will be 4 months with a follow-up period of 3 months.

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GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
BMI	Body Mass Index
BV	Bacterial vaginosis
CRF	Case Report Form
CRO	Clinical Research Organization
CT	Clinical Trial
ERC	Ethical Review Committee
GCP	Good Clinical Practice
IC	Informed Consent
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IS	Information Sheet
rERC	Reference Ethical Review Committee
SAE	Serious Adverse Event
STD	Sexually Transmitted Diseases
VAS	Visual Analogue Scale
VV	Vulvovaginitis
VVC	Vulvovaginal Candidiasis

2 INTRODUCTION AND JUSTIFICATION OF THE STUDY

Female lower genital tract infections are classified according to the primary location of signs and symptoms, theoretically distinguishing between vulvitis, vaginitis and endocervicitis. However, in practice, the inflammatory process is not usually limited to a single location, so in most patients it is manifested as vulvovaginitis (VV)[1].

VV, which can affect both the vaginal mucosa and vulvar skin, is typically associated to symptoms and discomfort such as leukorrhea, itching, stinging, dysuria and, in some cases, dyspareunia[2]. Most of these symptoms have an infectious origin, which is why their standardised approach involves the use of antifungal and/or antibiotic treatments[3].

According to different sources, VV represents more than one third of gynaecological consultations. Although most symptomatic episodes are effectively controlled with the different therapeutic alternatives available, the identification of pathogens resistant to the typically-used treatments[4] raises the need to look for new alternatives.

It is estimated that 20-25% of VV are caused by different yeast species belonging to the *Candida sp* family, with the most frequent genus being *C. albicans* (more than 80% of cases). Furthermore, bacterial vaginosis (BV), which causes 40-50% of VV, is associated with different microorganisms such as *Gardnerella vaginalis, Mycoplasma, Bacteroides, Mobiluncus, Peptostreptococcus,* among others[5].

Most of these microorganisms are part of the vaginal flora without clinically manifesting themselves. Evidence suggests that a change in the flora's balance, particularly in the population of acidifying bacteria in the vaginal environment such as *Lactobacillus*, influences the growth of another type of microorganisms that finally cause the patient to become symptomatic[6].

Recurring forms, more typical in the case of candidiasis (≥4 episodes/year) [7,8], usually occur during the 3 months after treatment[9]. The most accepted theory to explain this phenomenon is related to the persistence of the pathogen in the

vagina, corroborated by the isolation of forms with the same karyotype in recurrences[10-12].

Among the different treatment alternatives for infectious VV, the use of boric acid has been described as an effective, well-tolerated and particularly useful therapeutic option in the case of recurrent VV and candidiasis associated with non-albicans Candida[13,14]. Most studies with vaginal boric acid have studied its administration at doses of 600 mg[13,14].

Boric acid is considered a topical action agent that, by combining its bacteriostatic, fungistatic and fungicidal properties[15], has shown effective results in the control of infectious VV symptoms[16]. Although its mechanism of action is not clearly defined, a potential inhibitor effect of fungal oxidative metabolism that favours the properties previously described has been proposed[17].

In addition, many studies have been carried out in recent years that show positive results regarding the use of probiotics in patients with VV of infectious origin[18]. The use of these products in the treatment of infectious VV is based on the role of *Lactobacillus* as bacterial flora, maintaining a vaginal pH that promotes balance in the vaginal microenvironment, controlling the growth of potential pathogens that cause VV and reducing the possibility of overcolonisation by *Candida*[18]. There is little evidence available regarding the simultaneous use of antiseptics such as boric acid with probiotics to treat this type of patients.

De Seta and col. carried out a comparative study of conventional therapies for BV (Clindamycin) and VC (clotrimazole) and a combination of boric acid with probiotics (*Lactobacillus sp.*) in which a better concentration of lactobacilli was observed in the group treated with boric acid + probiotics 3 weeks after completing the treatment. These patients also registered a significant improvement in symptoms such as stinging, itching, the presence of leukorrhea and fetid vaginal discharge[19].

For all these reasons, the combination of boric acid with probiotics is postulated as a potential therapeutic alternative for the treatment of infectious VV, which

may end up being incorporated into the usual therapeutic arsenal against VV, and should therefore be compared to other current reference treatments in daily clinical practice.

3 OBJECTIVES OF THE STUDY

3.1 Main objective

To assess the effectiveness of the vaginal capsule with boric acid and *L. gasseri* and *L. rhamnosus* for the treatment of symptomatic episodes of vulvovaginitis (VV) against a control pharmacological treatment (according to the VV aetiology).

3.2 Secondary objectives

- To determine the incidence of recurrence/relapse at 3 months.
- To assess the level of post-treatment vaginal flora restoration.
- To characterise the different symptomatic and microbiological profiles of VV.
- To assess the tolerance and safety of the product.

4 SOURCE OF INFORMATION

The information will be obtained from the data collected during the patient's clinical visit, as well as from the data recorded by the patients and the self-administered scales of the Patient Questionnaires and Notebooks that will be handed to them by the investigators.

5 SCOPE OF THE STUDY

Outpatient gynaecology and national emergency clinics.

6 STUDY DESIGN

6.1 Type of study

Multicentric, open, prospective, drug-controlled, randomised pilot clinical trial in patients with vulvovaginitis of bacterial and candidal aetiology. Patients will be randomly assigned to two groups (healthcare product subject of study (boric

acid + *L. gasseri* and *L. rhamnosus)* or control treatment). The ratio will be 1:1 between the study product and the control treatment.

6.2 Study population

6.2.1 Inclusion criteria

- Women ≥ 18 years of age.
- Women with clinical manifestations of infectious VV.
- Women who agree to participate by signing the informed consent form.

6.2.2 Exclusion criteria

- Symptoms (high suspicion) compatible with infection due to *Chlamydia trachomatis*, *Trichomona vaginalis*, *Neisseria gonorrhoeae or Herpes simplex.*
- Use of antifungal or probiotic agents during the last 2 weeks prior to the study.
- Patients who are receiving any other treatment with probiotics, vitamin complexes that may significantly interfere with the study assessments.
- Pregnant patients.
- Patients who may become pregnant (if there is a reasonable doubt about the possibility of pregnancy at the time of inclusion or during the study a blood pregnancy test will be performed to rule out this possibility).
- Patients who are menstruating at the time of inclusion.
- Breast-feeding patients.
- Immunocompromised patients.
- Patients who, based on the investigator's criteria, are not expected to complete the follow-up.

6.2.3 Rescue criteria

In cases where the patient requires "rescue treatment", the treatments will be crossed. In the event of changing treatment group (crossover), the patient will be considered to be back at V_0 and the tests/examinations corresponding to the baseline visit will be repeated.

The patient will change group to receive alternative treatment in the following cases:

- Patient with hypersensitivity to the first assigned treatment.
- Worsening of symptoms within 3-4 days of starting the assigned treatment.
- Evidence or indications of lack of effectiveness after the first assigned treatment.
- Patient who demonstrates a willingness to change treatment.
- In cases where the perceived lack of effectiveness is associated with a suspected mixed aetiology infection (candidal and bacterial):
    - If they are in the control group, they will be changed to the treatment under study (boric acid + *L. gasseri* and *L. rhamnosus*).
    - If they are in the study group, they will be changed to the antifungal treatment (Gine-Canesten®; Clotrimazole 100 mg). If this treatment fails, BV will be treated with antibiotic treatment (Dalacin®; Clindamycin 100 mg).

6.2.4 Discontinuation criteria

- Patients who, during the study period, receive/use some type of treatment and/or intervention (probiotics, vitamin complexes or vaginal showers) that may significantly interfere with the study assessments.
- Patients who, even while receiving the rescue treatment, show a lack of effectiveness*. Patients who, after the baseline visit, are suspected to have an infection due to *Chlamydia trachomatis*, *Trichomona vaginalis*, *Neisseria gonorrhoeae or Herpes simplex.*

*These patients will also be subject to follow-ups during the 3 months of study.

6.3 Observation period

6.3.1 Inclusion period

The gynaecologist will choose the patients as they go to the clinic and meet the inclusion and exclusion criteria, according to the consecutive sampling technique. The assignment of the study treatment will be randomised,

depending on the previously-established code that will be assigned to each patient as they are included in the study. A recruitment period of 4 months and a follow-up period of 3 months are planned. Recruitment of participating centres will be competitive.

6.3.2 Follow-up period

Follow-up will be 3 months from the inclusion visit (V_0). Patients should attend two control visits in person and answer two telephone interviews.

6.3.3 Baseline Visit – V_0 (inclusion)

After the case history and initial examination, which will serve to substantiate the suspicion of VV due to infection, patients will be recommended to participate in the trial provided they meet the inclusion/exclusion criteria. Once the patient Information Sheet (PIS) has been handed over, the patient has signed the Informed Consent (IC) form and the investigator has verified that the patient meets the inclusion and exclusion criteria, they will proceed to fill in the CRF baseline visit sections.

During this first visit, socio-demographic data, relevant clinical history and gynaecological history will be recorded. The investigator will assess the patient's symptoms by recording the presence of clinical signs and symptoms of infectious VV (stinging, itching, erythema, oedema, abnormal vaginal discharge, etc.) and the *Sobel score*[20,21] *(see section 7.0).*The vaginal pH determination test will then be carried out and the samples for the different laboratory tests will be collected, which will consist of: spreading the vaginal discharge sample on a slide to subsequently count the *Lactobacillus spp.* and collecting the discharge sample with a swab that will also be used for the diagnostic confirmation culture and for the fresh vaginal smear. The samples will be stored properly according to the instructions specified in the manual of use of the sampling kit until the samples are collected.

The patient will be given the treatment corresponding to the code assigned to her, reminding her that she has the possibility of changing treatment group, at the time that she deems appropriate. If the assigned treatment is ineffective or

is not tolerable, the patient may contact the hospital to bring the first control visit forward and request a change of treatment.

During the baseline visit, the patient will be given a CRF which she must fill in and return at the end of the visit and a card with a link to the online home CRF (Patient diary) that must be filled in over the course of the study. The home CRF will be used to monitor the patient during the follow-up weeks. The home CRF may also be delivered on paper and the patient must deliver it later.

The investigator will give the patient product for one treatment cycle (which may range from 3 days (Clindamycin) to 7 days (boric acid 150 mg, *L. gasseri, L. rhamnosus*)).

The patient must return the empty blisters and/or surplus product during the first control visit. One week after the baseline visit, the patient will be contacted by telephone to find out her condition and if she is responding to the treatment, to assess the need for a rescue treatment or to arrange the first control visit. The first in-person control visit (V_1) will be carried out at least 2 weeks after the treatment has been completed.

6.3.4 Control telephone contact a week after starting the treatment:

The investigator will contact the patient and will proceed with a brief questionnaire to decide whether the patient responds correctly to therapy depending on the symptoms the patient will describe and their severity. The different options in this scenario are:

Patient condition	***Investigator's action***
Disappearance of symptoms	In-person appointment (V_1) within two weeks of the telephone contact and the end of the treatment (21 days from V_0).
Improvement of symptoms, but the patient still has mild clinical signs and symptoms	In-person appointment (V_1) within two weeks of the telephone contact and the end of the treatment (21 days from V_0); we recommend that she request an appointment if there is no improvement in the following days. If there is a suspicion that there is still an

	infection, rescue treatment will be recommended.
Deterioration/Lack of effectiveness	The patient must arrange an appointment as soon as possible to proceed with the alternative rescue treatment.

Depending on the investigator's criteria, an appointment will be made with the patient for an in-person visit. When making an in-person appointment, the investigator will take into account the date of the patient's last menstruation to prevent it from coinciding with the patient's 1st control visit. To this end, the investigator may slightly extend the 2-week margin previously established between the call and the control visit.

6.3.5 1st Control Visit (V_1)

The second in-person visit of the study will be carried out approximately two weeks after completing the treatment.

As with V_0, the investigator and the patient will have a CRF that they must fill in. The investigator will record the data regarding the clinical examination and repeat the tests and collect the samples corresponding to V_1. The results of the analytical tests carried out during V_0 will also be recorded.

The patient will fill in the corresponding CRF and return it at the end of the visit. An approximate time and date will be specified for the follow-up telephone interview around 2 months after the inclusion and an appointment will be made for the final follow-up visit (3 months from the patient's inclusion in the study).

If the investigator suspects a potential lack of effectiveness of the assigned treatment, rescue with the other therapeutic alternative of the study may be suggested according to the aetiology of the VV. In the event of crossing treatments, this visit will be considered the new baseline visit (V_0), for the purpose of collecting data and samples. The same procedure will be followed if the second visit is brought forward at the patient's request.

6.3.6 Telephone interview (TI) 8 weeks after the inclusion

Approximately 8 weeks after the baseline visit, the investigator will telephone the patient to interview her, with the main objective of knowing if there are signs of recurrence of VV.

The patient will confirm whether she is regularly filling in the online CRF and that she is not taking/using any products that may interfere with the assessment of the study objectives (*see section 6.2.4: Discontinuation criteria*).

The investigator will fill in a short form as a summary of the interview and confirm the appointment for the third and last in-person visit (V_2). The patient will be reminded that, if the home CRF is completed on paper, she must deliver it during the last visit.

6.3.7 2nd Control Visit (V_2)

Three months after the baseline visit, the patient will go to the last control visit, to assess the incidence of VV recurrences during the follow-up period. The investigator will perform, as in all visits, the corresponding case history and examination and will record data on clinical manifestations. This visit does not include taking samples for laboratory tests. If the patient has filled in the home CRF on paper, she must deliver it during this last visit.

6.4 Description of the intervention

The intervention will be assigned to each patient according to the code assigned by order of inclusion in the study and will be used for randomisation. In the case of the control group, the specific treatment will be indicated after randomisation depending on the aetiology of the VV:

1) **Candidal VV:**
    - **a.** Vaginal capsule with 150 mg of Boric Acid and Probiotics (*L. gasseri* and *L. rhamnosus*) plus prebiotics (1 capsule/24h for 1 week)
    - **b.** Clotrimazole vaginal tablet 100 mg/24h, 6 days (Gine-Canesten®)
2) **Bacterial VV:**

a. Vaginal capsule with 150 mg of Boric Acid and Probiotics (*L. gasseri* and *L. rhamnosus*) plus prebiotics (1 capsule/24h for 1 week)

b. Clindamycin vaginal suppository 100 mg/24h, 3 days (Dalacin®)

6.5 Randomisation

Patients will be randomly assigned to two groups according to the product under study or control product (two interventions considered as standard clinical practice based on the suspicion of aetiology associated to the VV). Random assignment of patients will be implicit in the code that will be assigned to them consecutively in each centre at the time of inclusion. The proportion of patients will be 1:1 between the study group and control group. Random lists will be generated with a software using blocks with up to three repetitions of the same treatment.

6.6 Concomitant treatments

The existence of concomitant treatments will be recorded. Only treatments that can influence the trial results will be reason for withdrawal of the product *(section 6.2.4)*.

6.7 Compliance with the intervention

Compliance with the indicated intervention will be checked with the data recorded by the patient in the home CRF, with direct questions during the second visit (first control visit) and empty blisters returned by the patient.

6.8 Pre-determination of sample size

A minimum of 50 patients are expected to be distributed between the study group (boric acid 150 mg, *L. gasseri, L. rhamnosus*) and control group (Clotrimazole 100 mg or Clindamycin 100 mg).

7 VARIABLES AND MEASURING INSTRUMENTS

7.1 Main assessment variables

- Presence and severity of clinical signs/symptoms associated to VV that the patient presents.
- Sobel score: Semi-quantitative scale where itching, erythema, oedema and stinging are indicated from 0 to 3: Absent (0), mild (1), moderate (2), severe (3)[20,21].
- Vaginal pH (to be determined by placing a vaginal discharge sample in contact with reactive pH indicator strips).
- Fresh vaginal smear (presence of "*clue cells*" or hyphas).
- Vaginal discharge sample culture.
- *Lactobacillus spp.* count in vaginal discharge.
- Relevant gynaecological history (including previous VV episodes).
- Compliance with the treatment and the gynaecologist's recommendations.
- Adverse Events.

7.2 Secondary assessment variables

- Socio-demographic data (age, marital status, etc.).
- Anthropometric data (height, weight, BMI).
- Relevant clinical history.
- Gynaecological history (surgery, STD, etc.).
- Current gynaecological situation (menopause, parity, stable partner, use of contraceptive methods).

7.3 End points

- Number of patients with VV recurrence during the follow-up period in each treatment group (incidence of recurrences after 3 months).
- Number of patients with resolved symptoms in each group 2 weeks after finishing the treatment.
- Changes in vaginal pH level in each group.
- Number of patients with "*clue cells*" or hyphas in the fresh smear.

- Profile and proportion of pathogen isolation in culture.
- Increase in the population of *Lactobacillus spp.* post-treatment.
- Rescue success rate in different scenarios.
- Relationship between compliance and treatment effectiveness.
- Relationship between compliance and recurrence after 3 months.
- Percentage of cases with good treatment tolerability.
- Rate of adverse events.
- Treatment tolerability.
- Proportion of patients with mixed infections (VC + BV).

8 STATISTICAL ANALYSIS

All data will be analysed using the SPSS-Windows statistical package.

8.1 Descriptive statistics

Descriptive statistics will be made of all the variables included in the CRF.

Categorical variables will be presented in the form of lists of frequencies and proportions. For quantitative variables (continuous or ordinal), central tendency (mean, medium, mode) and dispersion indices (standard deviation and maximum and minimum values) will also be presented.

8.2 Objectives of the study

The analysis of the main objective will focus on the comparison of the effectiveness of the product under study (boric acid + *L. gasseri* and *L. rhamnosus*) and that of the control treatment with solid vaginal forms, according to the usual clinical practice (clotrimazole or clindamycin) and assessed according to the patient's clinical symptom scores (VAS, Sobel *score*) approximately 2 weeks after the end of the treatment. To do this, T tests for independent samples or non-parametric Mann–Whitney U tests will be used.

For the secondary objectives, the level of restoration of vaginal flora will be assessed by comparing the Lactobacillus count in baseline discharges and 2 weeks after treatment through T tests for related samples or non-parametric

Wilcoxon or McNemar tests. The incidence of recurrences/relapses at 3 months will be studied by means of proportion comparison tests (Pearson's chi-squared test). The characterisation of the different symptomatic and microbiological profiles of VV, as well as the tolerability, compliance and adverse events, will be analysed through descriptive statistics.

The possibility of carrying out additional analyses, depending on the results, with the relevant statistical tests in each case is not ruled out.

9 ETHICAL CONSIDERATIONS

9.1 General considerations

This study must be carried out in accordance with the provisions of this protocol and the standards of Good Clinical Practice (GCP), as described in:

- ICH Harmonised Tripartite Guideline for Good Clinical Practice, 1996. Directive 91/507/EEC: Good Clinical Practice Guidelines for trials on medicinal products in the European Community.
- **Helsinki Declaration** in its latest revised version (Fortaleza, 2013) (Annex-1).

The study will only begin after obtaining written authorisation from the Ethical Review Committee of the Macarena-Virgen del Rocío University Hospitals in Seville, which act as a reference centre (rERC).

Except for emergency situations, changes or deviations from the protocol will not be permitted without documented approval.

The rERC shall be informed of any changes and shall approve in writing any changes or deviations that may increase the risks of the subject and/or may adversely affect the rights of the patient or the validity of the research. This stipulation does not apply to changes made to reduce inconveniences or prevent risks to subjects or to changes affecting the administrative aspects of the study (e.g. change of monitor).

9.2 Assessment of the risk-benefit for the research patients

In any case, the patient will receive a specific therapeutic alternative, in accordance with the diagnosis established by the investigator, so the suitability of the study treatments will not result in an increase in risk for the patient.

If the investigator or patient perceive a lack of effectiveness of the treatment, the patient may be rescued with the therapeutic alternative considered within the study.

9.3 Information sheet and informed consent (Annexes 1 and 2)

The investigator is responsible for ensuring that the patient (and/or legally authorised representative) understands the risks and benefits of her participation in the study, answering any questions that may be raised during the study, and sharing any new information that could influence her decision to continue to participate in the study in a timely manner.

The patient (or her legal representative) shall be informed of the characteristics of the CT, orally and in writing, by means of the PATIENT INFORMATION SHEET.

Finally, after being fully informed of the implications and restrictions of the protocol, having answered her questions and before starting the study, the patient will be asked to provide written INFORMED CONSENT. The patient information sheet model and the informed consent form are attached as an annex to this protocol. By signing and dating the informed consent form, the subject declares her voluntary participation and intention to comply with the Study Protocol and the Investigator Instructions and to respond to the questions raised during the Study.

The subject will keep the information sheet throughout the study, with all relevant study information, including the investigator's contact information. The investigator must keep the informed consent in the study file.

9.4 Review of the Ethics Committee

This protocol will be approved by the Ethical Review Committee of the Virgen Macarena-Virgen del Rocío University Hospitals in Seville, which will act as a reference committee for the other participating centres.

Any member of the Ethics Committee who has direct contact with this study as an investigator or as a member of staff of the centre will abstain from voting to approve the protocol.

All changes to the protocol will be specified as an amendment. The method for performing amendments will follow the standardised procedures according to RD223/2004.

9.5 Data confidentiality

By signing the protocol, the investigator agrees to keep all the information provided by the Sponsor in strict confidence and to insist on his team and the rERC keeping this confidence. The study documents provided by the Sponsor (protocols, investigator manual, CRFs and other materials) must be conveniently stored in the investigator's file, to ensure their confidentiality. The investigator will in turn ensure that the investigator's file is kept in accordance with the conditions specified in Directive 2001/20/EC.

The investigator will ensure the anonymity of the participants. Both in CRFs and other CT documents, subjects cannot be identified by their names but by an identification code. The investigator will keep a record of the inclusion of subjects showing the codes, names and contact information.

The information provided to the investigator by the Sponsor may not be disclosed to third parties without the direct written authorisation of the sponsor, except to the extent necessary to obtain the informed consent of the patients who wish to participate in this CT.

The information obtained will be considered strictly confidential on the basis of Organic Law 15/1999, of 13 December, on Personal Data Protection. (Official State Gazette No. 298, of 14-12-1999, p. 43088-43099) and its development

regulations. Medical data will be collected in accordance with Recommendation No. R (97) 5, of 13 February 1997, by the Committee of Ministers of the Council of Europe to the Member States on Medical Data Protection.

9.6 CRF records and reports

The investigator must ensure the accuracy, completion and relevance of the data communicated to the sponsor in the CRF and in all the reports requested. The data will be recorded in an online CRF to which only the investigator will have access through a user and password, both of which will be personal and non-transferable. All data obtained will be stored in an electronic file restricted to the staff of each centre and for which the principal investigator will be responsible.

Any change or correction to a CRF must be requested to the CRO responsible for monitoring, Clever Instruments S.L., by contacting the person responsible for the *data management* designated by the study sponsor. Changes must be documented and the investigator must keep records of changes and corrections.

The investigators or the institution must keep the CT documents, as required by applicable regulations. The investigator or institution must take measures to prevent the accidental or premature destruction of these documents.

At the request of the monitor, auditor, ERC or regulatory authority, the investigator/institution must have all records related to the CT available.

9.6.1 Monitoring Reports

The sponsor or CRO must send written summaries of the trial status to the rERC annually, or more frequently, if required by the rERC.

The sponsor or CRO must provide timely written reports to the rERC and, where relevant, to the institution on changes that significantly affect the development of the trial and/or increase the risk to the subjects. The investigator must supervise and sign these reports.

9.6.2 Safety Reports

The sponsor must be immediately informed of all serious adverse events, except those specified in the protocol or other document. These will be promptly reported in writing in detail and within the periods specified by the sponsor in the protocol. These follow-up reports must identify the subjects using the codes assigned in the CT and not by name, personal identification numbers and/or addresses.

9.6.3 Protocol Premature Termination or Suspension

If the study ends prematurely or is suspended for any reason, the investigator/institution must promptly inform the subjects of the study, providing them with appropriate therapy and follow-up. In addition:

- If the investigator terminates or suspends the study without prior agreement with the sponsor, the investigator must inform the institution where relevant, and the investigator/institution must promptly inform the sponsor and the ERC, as well as send a detailed written explanation of the termination or suspension to the sponsor and the ERC.
- If the sponsor terminates or suspends the study, the investigator must promptly inform the institution where relevant and the investigator/institution must promptly inform the ERC, sending a detailed written explanation of the termination or suspension.
- If the ERC withdraws the approval of the study, the investigator must inform the institution where relevant and the investigator/institution must promptly notify the sponsor and provide a detailed written explanation.

9.6.4 Final Investigator Report

When the study is completed, the investigator, where relevant, must inform the institution. The investigator/institution must provide the ERC with a summary of the study's results.

9.7 Monitoring

It is established that the study will be carried out at regular intervals. Monitoring will be carried out by the monitor(s) designated by the Sponsor. Regular contacts will be established between the Monitor(s) and the Investigator.

The Monitor will be responsible for verifying compliance with the protocol and the integrity, consistency and reliability of the data entered in the CRFs. To do this, the monitor must be able to access the participant's medical history and other documents. The investigator or his collaborators agree to cooperate with the monitor to ensure that the detection of any problem during the monitoring is resolved.

Extraordinary audits can be carried out to guarantee the validity of the study data and compliance with the regulations in force.

9.8 Study budget

The economic aspects of the study are detailed in the corresponding annex in a separate document.

9.9 Insurance

The sponsor of the study (Laboratorios Ordesa S.L.) will subscribe to an insurance policy that covers any damages that may be caused to the participants as a result of the study.

This insurance will also cover the responsibilities of the sponsor, the investigator and his collaborators and the head of the centre where the clinical study is carried out.

10 PRODUCT PREPARATION AND CONSERVATION

10.1 Conditioning

The product under study (vaginal capsule with 150 mg of boric acid, *Lactobacillus gasseri, Lactobacillus rhamnosus*.) must be received by the hospital pharmacy, which will be responsible for the control, distribution and

return of the product under research. Enough product for 70 patients will be assigned, taking into account that the study group must receive product for one treatment week (1 box) during the baseline visit.

10.2 CT sample, product or treatment labelling

As this is an open study, products administered to patients will not have any special labelling and will be labelled in the same way as they are usually on the market.

10.3 Conservation, distribution and return

Products provided for the CT must be stored at room temperature in a restricted area free from extreme environmental conditions. This should be an area that can be closed, with access limited to the investigator.

When received, an inventory of the shipment must be made. The document must be signed by the investigator or authorised person and a copy will be sent to the Monitor and another to the Investigator's Archive.

The investigator will be responsible for the return of the products, and will document any discrepancies in the formula stock records.

11 ADVERSE EVENTS

The Adverse Events (AE) that appear during the study will be assessed. An AE is any harmful event in a patient after randomisation and during a study, even if it is considered not related to the treatment of the study. ***In this CT, AEs will be collected and recorded in the CRF, provided that they are serious (if they cause clinical signs or symptoms and are considered clinically significant) and/or are related to the treatments under study.***

Diseases or medical conditions that were present before starting the medication under study will only be considered AEs if there is a worsening after the start of the study treatment (any procedure specified in the protocol).

As far as possible, AEs will be described according to:

- Duration (start and end dates).
- Degree of severity (mild, moderate, severe).
- Relationship with medication under study (suspected/not suspected).

- Action(s) taken to correct it.

11.1 Types

Any adverse and unintentional sign (abnormal and clinically significant analytical result) associated or not to the use of the drugs will be considered an adverse event.

11.2 Clinical signs secondary to the use of the study drug

i. Vaginal itching sensation[13]
ii. Water discharge during treatment[13]
iii. Vaginal erythema[13]
iv. "Sandy" sensation during sexual intercourse[14]
v. Acute systemic toxicity due to boric acid[14]
vi. Chronic toxicity due to the use of boric acid[14]
vii. Bacteraemia caused by *Lactobacillus spp.*[17]

11.3 Serious Adverse Events

Information on Serious Adverse Events (SAE) and Unexpected Serious Adverse Reactions (USAR) with **suspicion of causal relationship** with the treatments under study will be collected and recorded in the Serious Adverse Events notification form (RD223/2004). These serious adverse events must also be notified to the Spanish Agency of Medicines and Medical Products (AEMPS). The AEMPS will be notified by the ESIC, for which reason the investigator must notify them within 24 hours of knowing. Only serious, unexpected AEs related to treatments under study will be reported to the AEMPS.

Any adverse event or adverse reaction is considered to be a SAE or USAR when, at any dose, it:

• Leads to the death of the patient,
• Threatens the life of the subject,
• Forces the subject to be hospitalized or to extend their stay,
• Leads to permanent or significant disability, or
• Results in a congenital anomaly or malformation.

The intensity of the AE will be classified as mild, moderate or severe. The investigator will record the relationship of the study product with the AE according to the following causality terms:

- Related: The AE follows a reasonable time sequence from the moment of exposure to the product. It cannot be explained by the subject's clinical condition or by the study procedures/conditions. The AE decreases after the discontinuation of the study product and reappears once the study product is re-administered.
- Potentially related: The AE follows a reasonable time sequence from the time of exposure to the product, but may have been caused by the subject's clinical condition or by the study procedures/conditions.
- Unlikely related: The temporary association between the AE and the study product is such that it is unlikely that there is any reasonable relationship between them. The relationship is not likely due to other possible explanations.
- Not related: The AE has undoubtedly been caused by the subject's clinical condition or by the procedures/conditions of the CT. A reasonable explanation must be given, for example, that the product has not been consumed under research or an incompatible temporary relationship.
- Not assessable: The report indicating an adverse reaction cannot be judged because the information available is insufficient or contradictory, and no extra data can be added or verified.

11.4 Action taken and resolution of AE

The investigator will record the action(s) taken with the product and the effects of the event for each of the AEs, according to the following:

- Action taken in relation to the product
- Resolution

11.5 Follow-up of patients with AE

Patients can be withdrawn from the CT at any time. Patients who have been withdrawn from the study due to an AE must be monitored by the investigator until the clinical result of the AE is determined.

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