Title of the study	Prospective study to evaluate the effect of a probiotic on microbiota composition and inflammation in women with endometriosis
Study code	ENDO-1
Promoter	CHEMO Research S.L.
Center	
Principal Investigator	

1. INTRODUCTION

We are writing to inform you about a research study in which you are invited to participate. This study has been approved by the Ethics Committee for Research with Medicines of the Hospital La Paz, in accordance with current legislation and the Law 14/2007 on Biomedical Research.

Our intention is that you receive correct and sufficient information so that you can decide whether or not to participate in this study (participation is completely voluntary). To this end, before making a decision about your participation, please read this information sheet carefully and ask any questions you may have; we will clarify any doubts you may have. You are also welcome to consult with others before making your decision.

If after having read it and clarified your doubts with the research staff you wish to participate in the study, you will be asked to sign the Informed Consent Form at the end of this document and you will be provided with a copy of it.

2. VOLUNTARY PARTICIPATION

You are invited to participate in the study because you have been diagnosed with endometriosis. You should know that your participation is voluntary and that you may decide NOT to participate. If you decide to participate, you can change your decision and withdraw your consent at any time, without altering your relationship with your physician or harming your health care.

3. OBJECTIVE OF THE STUDY

Endometriosis is a complex and very common gynecological disease that affects approximately 10% of women of reproductive age. While there are women with

Endometriosis is a disease that can have a great impact on the physical, mental, sexual and social well-being of the woman who suffers from it, due to manifestations such as pain, which can become very intense and disabling, in addition to endometriosis being an important factor causing infertility.

To date there is no cure for endometriosis, the available treatments (surgical and pharmacological) are aimed at controlling the symptoms and not at curing the disease. The role of the human microbiota (a set of microorganisms that are normally found in the human body) in the development and evolution of endometriosis opens up a new avenue for the treatment of this disease. This study aims to test the effect of modifying the microbiota, favoring the growth and expansion of beneficial bacteria to the detriment of potential pathogens, through the direct administration of live bacteria, called probiotics.

The main objective of this study is to evaluate the effect of a probiotic strain on the composition of the vaginal microbiota and inflammation in women with endometriosis, to ultimately test the impact, as an adjuvant to regular hormone therapy, on the quality of life of patients.

4. DESCRIPTION OF THE STUDY.

This study will be conducted in 40 women, between 18 and 45 years of age, who have been diagnosed with endometriosis.

Each study participant will be randomly assigned to one of two different treatment groups in the study and will receive either the probiotic or placebo (placebo being a capsule that looks like the probiotic but contains no pharmacologically active substance and therefore is not expected to have any effect), along with an oral contraceptive. You will be equally likely to be treated with either the probiotic or the placebo. The decision whether you take the probiotic or the placebo depends solely on chance (like flipping a coin) and neither you nor your doctor will know which treatment you are taking during the course of the study.

Regardless of the treatment group to which you are assigned, in addition to the investigational product (probiotic or placebo), you must take a daily progestin contraceptive (Dienogest 2 mg).

On the other hand, for the treatment of pain, you may also receive analgesics or non-steroidal anti-inflammatory drugs (NSAIDs) according to medical indications.

5. STUDY ACTIVITIES

Your participation in the trial will last approximately 4 months, counting from the time you start treatment, during which you will have to come 2 times to the study site, firstly, to the screening visit, where you will be informed about the study and, if you wish to participate, you will have to sign the informed consent form at the end of this document. During this visit it will also be determined whether you meet the criteria for participation and, if so, you will be given the treatment/investigational product and begin your participation in the study (day 1). At the end of treatment, day 112-week 16, you will need to come to the center a second time.

Additionally, the first week of each month, until the end of the treatment (week 16), you will receive a telephone call from your physician to follow up on your pain level, relevant changes in your health status (adverse events), concomitant medication and treatment compliance (probiotic or placebo intake, depending on the treatment group to which you belong, and contraceptive). These data should be collected daily in the patient's diary, which will be given to her during visit 1 and in which she will also record the daily level of pain.

At both visits at the center, you will be given a pregnancy test. In addition, your physician will perform a physical/gynecological examination and ask you about your general health and medication intake. During these visits, a blood sample will also be taken to determine the levels of certain sex hormones, as well as a vaginal swab. In addition, you will be asked to indicate your level of pain and complete a simple questionnaire on your state of health, which will be used to assess the evolution of the disease.

During the 4 months of your participation in the study, you should take 1 tablet of the investigational product (probiotic/placebo) daily by mouth about 30 minutes after dinner (before going to bed) provided by your doctor, in addition to 1 tablet of Dienogest 2 mg that you usually take or are going to start taking as a basic treatment for endometriosis (following the schedule indicated by your doctor). For the duration of your participation, you should not interrupt or modify the dose of the treatment, except in cases of urgency, adverse events or your doctor's recommendation.

During your visits to the center you will need to perform the procedures described below in the study schedule:

Procedures	Visit 1 Selection / Start of treatment	Monthly call	End-of-study visit
	V1		V2
	Day 1	Day 1-7 of each month	Day 112 ± 3
Signature of consent informed	X		
Criteria check of selection	X		
Assignment of the group of treatment (randomization)	X		
Medical history	X		
Taking of vital signs (T[a], blood pressure and pulse)	X		X
Exploration physical/gynecological	X		X
Sample extraction from blood	X		X
Sampling of vaginal exudate	X		X
Pregnancy test (urine sample)	X		X
Pain assessment (NRS)	X		
Revision of the register of pain assessment (NRS)		X	X
Endometriosis Health Profile (EHP-30)	X		X
Registration of adverse events	X	X	X
Medication review concomitant	X	X	X
Product dispensing under study	X		
Product count consumed			X
Revision of the patient		X	X

All the procedures you will undergo are part of the usual clinical practice for the follow-up and treatment of your disease, with the exception of pregnancy tests and questionnaires to be filled out together with the extraction of blood samples to determine hormone levels and inflammatory factors associated with endometriosis and vaginal exudate to analyze the vaginal microbiota.

The biological samples collected (blood, urine, vaginal exudate) will be used only for the purposes of this assay, will be kept for the necessary time and will be destroyed at the end of the assay. Your samples will not be stored or used for purposes other than those indicated. Throughout this process, the principal investigator of the project will be responsible for the samples.

The blood samples will be analyzed in the laboratory of the Clinical Analysis Service of the Hospital Universitario La Paz and the vaginal exudate samples will be analyzed by a laboratory of the Universidad Complutense de Madrid.

The treatment of the biological samples obtained in this research will be in accordance with the provisions of the Biomedical Research Law 14/2007 and Royal Decree 1716/2011, of November 18, which establishes the basic requirements for the authorization and operation of biobanks for biomedical research purposes and the treatment of biological samples of human origin, and regulates the operation and organization of the National Register of Biobanks for biomedical research.

By signing the attached consent form, you agree to comply with the study procedures that have been explained to you.

The investigator may decide to terminate the study early or discontinue your participation, for safety reasons or non-compliance with study procedures. In any case, you will be informed of the reasons for your withdrawal.

6. RISKS AND DISCOMFORTS ARISING FROM THEIR PARTICIPATION IN THE STUDY

The product that is the subject of this study complies with all the legal requirements of composition and safety for its use in humans. There is currently no information available to indicate that the consumption of this supplement, at the established doses, can cause any harm to health.

On the other hand, you should know that the contraceptive to be taken daily together with the investigational product (Dienogest 2 mg) is a drug authorized for the treatment of endometriosis in Spain. As with all medications, taking Dienogest may cause, in some people, the appearance of adverse reactions. The possible side effects will be described in its data sheet.

On the other hand, during the study, some samples are obtained that will determine whether or not you can participate in the study (pregnancy test at the initial visit, among others) and that will make it possible to evaluate your disease. The risks associated with the procedures used to obtain them are explained below:

- **Urine sample:** a sample will be obtained at visit 1 of the study in order to rule out pregnancy and at visit 2 to confirm that pregnancy has not occurred. You will be asked to urinate into a small cup. This test will not cause you any discomfort.

- **Blood samples:** 2 samples will be obtained throughout the study (1 at each of the study visits) and the amount drawn for each analysis will be

approximately 10 ml of blood, similar to what is obtained in a routine analysis. These samples are obtained for the purpose of determining levels of inflammatory factors and hormone levels. For most people, needle punctures for blood collection are not a problem. However, they can cause bleeding, bruising, discomfort, infection and/or pain at the site of blood collection. You may also feel dizzy.

- **Vaginal exudate sample:** 2 samples will be obtained throughout the study (1 at each of the visits) in order to determine the composition of the vaginal microbiota. This sample will be taken by your doctor, simply through the introduction of a sterile cotton swab into the vagina to take a sample of secretion, so it is a completely painless procedure, although for some women it may be somewhat uncomfortable.

If you decide to participate in the study, you will be expected to attend each scheduled visit, within the stipulated time, and to perform the procedures outlined in the previous section. In addition, you will be asked to inform the research team of any events that occur during your participation. Regarding the control of the dietary supplement, you will be asked to fill out diaries in which you should indicate the times you take it and any changes related to your health, so that any alteration that affects you in a negative way will be reflected.

7. POTENTIAL BENEFITS

Although there are no previous studies evaluating the effect of this bacterium, used as a probiotic in this study, on endometriosis disease, it has been shown in previous clinical trials to have the ability to modulate immune response and inflammation, influence estrogen levels and microbiota composition, promote healthier microbial communities, and exert therapeutic effects in various diseases; this species demonstrated its ability to increase the likelihood of successful pregnancy in women with a history of infertility and recurrent miscarriages.

Therefore, there is a possibility that you may not derive any health benefit from participating in this study. The data obtained during the study will be used to gain further knowledge about the course and treatment of endometriosis.

8. CONTACT AT CASE AT DOUBTS/INCIDENTS O INFORMATION RIGHTS

If during your participation you have any questions or need further information, please contact _____ from the Gynecology and Obstetrics service of the _____through:

E-mail: Phone:

9. PREGNANCY

There is currently no data to indicate that the consumption of this product may be harmful to the health of pregnant women or the fetus. However, a pregnancy test will be performed prior to your participation in the study to ensure that you are not pregnant. Throughout the study, you must follow a course of oral contraceptives containing a progestogen, in this case Dienogest 2 mg, for the duration of the study and continue until the end of the indicated treatment.

If pregnancy occurs during your participation in the study, you should inform your physician immediately so that you can receive appropriate medical care. If you become pregnant, your participation in the study will be terminated. In addition, you would be asked to collect data on the evolution of your pregnancy and health data of the baby at the time of delivery, always ensuring compliance with the Organic Law 3/2018, of December 5, on the Protection of Personal Data and Guarantee of Digital Rights.

10. ALTERNATIVE TREATMENTS

As previously mentioned, to date there is no cure for endometriosis; the treatments available, whether pharmacological and/or surgical, are usually aimed at controlling the symptoms and not at curing the disease. Among the most commonly used pharmacological treatments are steroid contraceptives, a combination of birth control pills, progestins and GnRH analogs to modulate sex hormone levels, and non-steroidal anti-inflammatory drugs and analgesics for pain. Surgical treatment aims to remove the lesions, adhesions and scar tissue of endometriosis.

The probiotic in this study will be administered in combination with a progestogen contraceptive, Dienogest 2 mg, commonly used for the treatment of endometriosis.

In case you decide not to participate in the study, your physician will explain to you the most convenient therapeutic options for the treatment of your disease.

11. PROTECTION OF PERSONAL DATA

All your data collected during the trial will be kept strictly confidential, and will be treated by both the Promoter and the Center in accordance with the provisions of the General Data Protection Regulation (EU) 2016/679 ("GDPR") and the Spanish Law 3/2018, of December 5, on the protection of personal data and the guarantee of digital rights ("LOPDGDDD").

Your data are necessary for the sponsor to conduct this research, develop the drug, and study the trial disease. Therefore, your data will be used in this study as well as within related research activities in order to: (i) understand how the study drug works in the body; (ii) better understand the studied disease and associated health problems; (iii) learn from previous studies to plan new studies or improve scientific analysis methods; (iv) publish research results in scientific journals or use them for educational purposes. For more information about confidentiality and the treatment of your personal data, please refer to **Appendix I** attached to this document.

12. EXPENSES AND FINANCIAL COMPENSATION

The study sponsor is responsible for managing the supply of the investigational product and the financing of the trial. In order to carry out the trial, the sponsor has signed a contract with the principal investigator of the trial and the center where the trial is to be carried out, where the economic compensation received by the center for carrying out the trial is established.

It is possible that commercial products or patents may be derived from the results of the study; in this case, you will not participate in the economic benefits originated. However, your participation in the study will not entail any costs for you since you will receive the product under investigation free of charge and €50 as compensation for your expenses in attending each of the visits scheduled at the center, so that if you complete the study (completion of the 2 on-site visits) you will be compensated with a total of €100.

13. WHAT TREATMENT WILL I RECEIVE AT THE END OF THE CLINICAL TRIAL?

The product object of this research is not a drug, it is a bacterial strain, of the species *Lactobacillus salivarius* (a probiotic). At the end of your participation, you will receive

the best treatment available and that your physician considers the most appropriate for your disease. Neither the investigator nor the sponsor makes any commitment to continue supplying the investigational product (the probiotic) outside of the study.

14. OTHER RELEVANT INFORMATION

You should be aware that any new information regarding the probiotic to be used in the study, which may affect your willingness to participate in the study and which is discovered during your participation in the study, will be communicated to you by your physician as soon as possible.

You may be excluded from the study if the study sponsor or investigator deems it appropriate, either for safety reasons, because of any adverse events that occur, or because they feel that you are not complying with established procedures. In either case, you will receive an adequate explanation of the reason for your withdrawal from the study.

Whether you decide to leave the study prematurely or the investigator decides to discontinue your participation, the data collected up to that point may be used only for the purpose of the study, but no new data will be collected.

Thank you very much for your cooperation.

CONSENT DOCUMENT FOR PARTICIPATION IN A RESEARCH STUDY

TITLE: Prospective study to evaluate the effect of a probiotic on microbiota composition and inflammation in women with endometriosis.

Me, _____

☐ *I have read the participant information sheet for the above-mentioned study that was given to me. given to me, I was able to chat with . with:*
_____ *y make all questions about the study as needed.*

☐ *I had enough time to decide if I wanted to participate.*

☐ *I understand that my participation is voluntary, and that I can withdraw from the study whenever I want, without having to give explanations and without repercussions on my medical care.*

☐ *I agree to the use of my data and samples under the conditions detailed in the participant information sheet.*

☐ *I agree to have my primary care physician informed of my participation in the study.*

☐ *I freely agree to participate in this study.*

☐ *I receive a signed and dated copy of this informed consent document.*

S.D.: The participant, S/he researcher

First and last name: _____ First and last name: _____

Date: Date:

WITNESSED CONSENT FORM FOR PARTICIPATION IN A RESEARCH STUDY

TITLE: Prospective study to evaluate the effect of a probiotic on microbiota composition and inflammation in women with endometriosis.

I,

_____

as an impartial witness, affirm that in my presence:

- ☐ *The above-mentioned study participant information sheet has been read to __the above-mentioned study participant information sheet given to him/her and was able to ask all necessary questions about the study.*
- ☐ *He has had enough time to decide if he wanted to participate.*
- ☐ *He has understood that his participation is voluntary, and that he can withdraw from the study whenever he wants, without having to give explanations and without this impacting his medical care.*
- ☐ *You agree to the use of your data and samples under the conditions detailed in the participant information sheet.*
- ☐ *You agree that your primary care physician will be informed of your participation in the study.*
- ☐ *He freely agrees to participate in this study.*
- ☐ *You receive a signed and dated copy of this informed consent document.*

Signature of the witness

S/he signature of the investigator

First and last name: _____

First and last name: _____

Date:

Date:

APPENDIX I. PERSONAL DATA PROTECTION INFORMATION

Who is the Data Controller?

Both the Center and the Sponsor are responsible for their respective treatments, and each of them is responsible for the obligations derived from their activity. The Center is responsible for all the data contained in the clinical history that can identify you, and the Sponsor for the data collected in this study in coded form (pseudonymized). The role of the data controller is to ensure that your information is used correctly and that appropriate security measures are implemented in accordance with the regulations.

The Promoter and the Center, as Data Controllers, guarantee that they comply with the provisions of the EU Regulation 2016/679 General Personal Data Protection Regulation and the Organic Law 3/2018 General Law on Data Protection and Guarantee of Digital Rights.

What are your personal data used for and what is the basis of legitimacy?

(i) Activities related to the protocol of this trial: Your personal data will be processed, on the one hand, to ensure the reliability and security of the processing subject of the trial, in compliance with the legal obligations of the Sponsor and the Facility. The legal basis for the processing is Article 6(1)(c) of the GDPR, with the processing of sensitive data being lawful in accordance with Article 9(2)(i) for reasons of public health interest and to ensure the quality and security of the trial processing. Examples of such processing activities are: the obligation of security notification to the competent authorities, keeping the trial master file, or the disclosure of trial data to the competent authorities in the framework of an inspection.

On the other hand, your data will be processed in order to carry out the research of the present trial. Irrespective of the informed consent provided for healthcare purposes, the processing activities involved in this research are covered by the public interest, Art. 6.1. (e) GDPR, and by the legitimate interest of this research, Art. 6.1. (f) GDPR, when the particular activity does not pursue a public interest purpose. The processing of health data is lawful under Article 9.2 (i) and (j) of the GDPR, for reasons of public health interest and being necessary for the research.

(ii) In addition, the Center will process your personal data for health care and health treatment purposes, including your medical history.

(iii) The biological samples collected will be used only in the present trial and will be destroyed after the final publication of the trial.

What about confidentiality?

The data obtained during the trial will be identified by a random code assigned to each patient without including personal information. The information that associates the code to your identity will be kept exclusively by the Center, being possible only for the study physician to relate such data to you and your medical history, and the Sponsor or third parties will not be able to identify you directly unless legally required to do so.

Which third parties can access your personal data?

When necessary for the management of clinical research, third parties may have access to personal information in addition to the physician or investigators conducting the study, such as professionals of the research team and personnel authorized by the Sponsor (study monitors, auditors) to check the study data and procedures. The confidentiality of the accessible data will be maintained in all cases.

To whom is your data communicated?

For the fulfillment of the Sponsor's legal obligations in research, your data may be communicated to health authorities, the Research Ethics Committee and insurance companies in the framework of an inspection or for adverse reaction management. In addition, your pseudonymized personal data may be transferred to international subsidiaries of the Insud Pharma Group, both within and outside the European Economic Area ("**EEA**"). In case your data, even pseudonymized, is transferred outside the EEA, the Promoter will implement the measures required by the GDPR. You can find out about the Promoter's subsidiaries at www.insudpharma.com/donde-estamos

How long will your personal data be kept?

Your data will be kept for a period of 25 years after the end of the trial. Subsequently, they will be retained by the Center to ensure adequate health care.

How can you exercise your data protection rights?

You may exercise your rights of access, rectification, erasure, objection, limitation of processing and portability of your personal data, subject to the limitations established in the Good Clinical Practices and applicable legislation, by contacting the Center or the Promoter. If you exercise your right to object or revoke this Informed Consent, you will no longer participate in the study. However, in order to guarantee the scientific integrity of the

trial, your personal data already held will continue to be processed in accordance with the purposes described and applicable law. Your decision not to continue in the trial will not result in any penalty or loss of access to medical treatment or health care entitlements. In addition, the competent authority or the Sponsor may terminate the study without your consent, in which case you will be informed of the reason for termination.

How can I contact the Center and the Developer?

You can contact the Center at [*contact email address] or the Promoter's Data Protection Officer at dataprotection@insudpharma.com

Where will the results be published?

Upon completion of the study, the anonymized results will be published in scientific journals and on the internet (www.clinicaltrials.gov and www.clinicaltrialsregistry.eu). Your physician will provide you with information regarding the trial results once available.