

## ENDOMETRIAL MICROBIOME METAGENOMIC ANALYSIS (EMMA)

Patient information		Sample information		Clinic information	
Unique pat id:	781966	Date received:	12/09/2024	Clinic:	FERTILITY ASSOCIATES WELLINGTON
Patient name:	KATRINA MURISON	Report date/time:	12/23/2024	Clinician:	Dr. LEIGH SEARLE
Patient DOB:	12/06/1988	Sample type:	Endometrial Biopsy		
Allergy to antibiotics:	No	Cycle type:	HRT		
		Cycle day:			
		No. Biopsy:	1		
		Date of biopsy:	11/30/2024		

### RESULTS OF EMMA TEST:

LACTOBACILLUS			
BACTERIA	RESULT	VALUE	REFERENCE RANGE
<i>Lactobacillus crispatus</i>	Not detected	N/A	≥ 3.71
<i>Lactobacillus gasseri</i>	Not detected	N/A	≥ 3.60
<i>Lactobacillus iners</i>	Not detected	N/A	> 3.57
<i>Lactobacillus jensenii</i>	Not detected	N/A	≥ 3.70
PATHOGENS OF THE REPRODUCTIVE TRACT			
<i>Actinomyces israelii</i>	Not detected	N/A	Absent
<i>Atopobium vaginae</i>	Not detected	N/A	≤ 3.57
<i>Bacteroides fragilis</i>	Not detected	N/A	≤ 3.57
<i>Bifidobacterium spp</i> †	Not detected	N/A	≤ 4.22
<i>Clostridium sordellii</i>	Not detected	N/A	Absent
<i>Fusobacterium nucleatum</i>	Not detected	N/A	Absent
<i>Gardnerella vaginalis</i>	Not detected	N/A	≤ 3.72
<i>Haemophilus ducreyi</i>	Not detected	N/A	Absent
<i>Mycobacterium tuberculosis</i>	Not detected	N/A	Absent
<i>Mobiluncus spp</i>	Not detected	N/A	≤ 3.57
<i>Peptostreptococcus anaerobius</i>	Not detected	N/A	≤ 3.57
<i>Porphyromonas asaccharolytica</i>	Not detected	N/A	≤ 3.57
<i>Prevotella bivia</i>	Not detected	N/A	≤ 3.57
<i>Prevotella disiens</i>	Not detected	N/A	≤ 3.57
<i>Sneathia spp</i>	Not detected	N/A	≤ 3.57
<i>Treponema pallidum</i>	Not detected	N/A	Absent

†Bifidobacterium spp, when detected without other pathogens, could be displaced from its niche using probiotics.

### RECOMMENDATION

Probiotic treatment with Lactobacillus (preferably vaginal) as stated by the manufacturer's recommended dose, could help to increase the amount of this bacteria in the reproductive tract.

The analysis of a new biopsy following probiotic treatment is not required.

### RESULTS OF ALICE TEST :

PATHOGENS RELATED TO CHRONIC ENDOMETRITIS			
BACTERIA	RESULT	VALUE	REFERENCE RANGE
<i>Chlamydia trachomatis</i>	Not detected	N/A	Absent
<i>Enterococcus faecalis</i>	Not detected	N/A	≤ 3.63
<i>Escherichia coli</i>	Not detected	N/A	≤ 3.58
<i>Klebsiella pneumoniae</i>	Not detected	N/A	≤ 3.57
<i>Mycoplasma genitalium</i>	Not detected	N/A	≤ 3.57
<i>Mycoplasma hominis</i>	Not detected	N/A	≤ 3.57
<i>Neisseria gonorrhoeae</i>	Not detected	N/A	Absent
<i>Staphylococcus aureus</i>	Not detected	N/A	≤ 3.57
<i>Streptococcus agalactiae</i> group B/ <i>Streptococcus viridans</i>	Not detected	N/A	≤ 3.57
<i>Ureaplasma urealyticum</i>	Not detected	N/A	≤ 3.58

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## INTERPRETATION OF EMMA RESULTS

No DNA from reproductive tract potentially pathogenic bacteria, not related to chronic endometritis, has been detected.

No DNA from *Lactobacillus* has been detected in the endometrial sample. *Lactobacillus* is the predominant bacteria in the female reproductive tract at reproductive age. It is not necessary to have different *Lactobacillus* strains but at least one of them should be within the range established as reference values.

## INTERPRETATION OF ALICE RESULTS

No DNA from potentially pathogenic bacteria related to chronic endometritis has been detected.

## COMMENTS

None

## TEST DESCRIPTION

EMMA (Endometrial Microbiome Metagenomic Analysis) is a molecular microbiology tool used to determine whether the uterine microbial environment is compatible with that which, according to what has been described in the scientific literature (1,2,3) is more favourable for achieving pregnancy. This molecular method is based on the detection and measurement of the amount of bacterial DNA present in a sample of endometrial tissue. EMMA helps to infer whether the endometrium presents a *Lactobacillus*-dominated flora.

The microorganisms analyzed in this test are: 4 *Lactobacillus* species (*L. crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*) and 16 reproductive tract pathogens: *Actinomyces israelii*, *Atopobium vaginae*, *Bacteroides fragilis*, *Bifidobacterium* spp, *Paeniclostridium sordellii* (formerly *Clostridium sordellii*), *Fusobacterium nucleatum*, *Gardnerella vaginalis*, *Haemophilus ducreyi*, *Mobiluncus* spp, *Mycobacterium tuberculosis* complex, *Peptostreptococcus anaerobius*, *Porphyromonas asaccharolytica*, *Prevotella bivia*, *Prevotella disiens*, *Sneathia* spp and *Treponema pallidum*.

EMMA also always includes the ALICE test (Analysis of Infectious Chronic Endometritis), used to detect the presence of DNA from the bacterial pathogens most frequently related to chronic endometritis (CE), a subclinical infection of the endometrium that has been associated with infertility, especially to repeated implantation failure and recurrent pregnancy loss. This molecular method is based on the detection and measurement of DNA from 10 bacterial pathogens often related to Chronic Endometritis, such as: *Chlamydia trachomatis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Streptococcus agalactiae* (group B) & *Streptococcus viridans*, and *Ureaplasma urealyticum*.

Therefore, EMMA&ALICE provide information on the presence/absence of DNA from disease-causing pathogens (culturable and non-culturable) to help the physician guide and personalize treatment, if deemed necessary, for patients with suspicion of asymptomatic chronic endometritis or other reproductive tract infections.

## TESTING METHODOLOGY

EMMA is a molecular test which utilizes RT-PCR to provide microbiota information in endometrial tissue by analyzing a customized panel of bacteria including information about the most frequent *Lactobacillus* species and potentially pathogenic bacteria of the reproductive tract (some of them related to with chronic endometritis).

The technology used for these purposes is based on DNA extraction followed by microorganism-specific amplification that enables the quantification of targeted bacteria present in a sample. After receiving the endometrial biopsy and extracting the genetic material (DNA), sample minimum quality requirements are evaluated before using the screening tools.

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## TEST LIMITATIONS

The aim of this test is to provide physicians with an objective molecular detection of the patient's endometrial microbial health, including pathogens causing CE. Depending on the result of this analysis, the physician may use it to improve uterine microbial health.

The results of the EMMA test may vary depending on different factors such as: hormonal changes, antibiotics intake, sexual activity, hygiene habits, etc. The result of the EMMA test does not exclude the presence of DNA from other microbial pathogens in the uterine cavity than the ones detailed in the Test Description section above.

Detection of a microorganism by this test does not imply a diagnosis. Similarly, a negative result does not rule out the possibility of disease.

Following the recommendations indicated in this report does not guarantee a successful pregnancy. Implantation failure may be caused by other factors such as: a shift in the implantation window, poor embryo quality, genetic anomalies or other pathologies.

This test is not aimed at diagnosing sexually transmitted diseases. If there is suspicion of this type of disease, or if the patient belongs to a risk group for these diseases, we recommend that you consult an infectious disease specialist to offer the patient an adequate diagnosis and treatment.

## LEGAL/QUALITY

IGENOMIX (part of Vitrolife group) will only release the report once a completed Test Requisition Form is received. The clinic/clinician/certified health professional requesting the test is responsible for obtaining and taking custody of "Informed Consent" from the patient as depicted by national guidelines and/or legislation.

## EXEMPTION CLAUSE OF DIAGNOSTIC LIABILITY

The prescribing physician shall have a provider-patient relationship with each patient referred for this test and be solely responsible for all clinical diagnosis and treatment of his/her patients, including determining those patients that may benefit from this test. The prescribing physician acknowledges that Igenomix does not exercise control or direction over, and shall not be responsible for, the means, methods, or manner by which the prescribing physician exercises professional judgment in the provision of medical care to its patients, including the referral of patients for this test and including any advice that the prescribing physician may provide, and any decisions that patients may accordingly make, based on the results. The prescribing physician acknowledges and shall advise its patients for whom Igenomix provides its services, that the tests are inherently probabilistic in nature, and, accordingly, that a favorable test result is not, and cannot be, a guarantee of the achievement of a successful implantation, pregnancy, or other desired outcome. Even tests that are performed with due care can, from time to time, produce inaccurate results, for which neither Igenomix, nor any other Labs, nor any other person, shall have any liability. It is essential that all patients receive appropriate counselling concerning the tests and their results. The prescribing physician shall, to the extent appropriate in the reasonable exercise of good medical judgment, provide that counselling to its patients or to arrange for that counselling through an appropriate third party. The prescribing physician shall ensure that the objectives and limitations of the tests are thoroughly discussed with the patient. It should be emphasized that methods for the genetic analysis of samples are not, and cannot be, 100% accurate. In particular, prescribing physician will ensure that the patients understand that the purpose of the test is not to guarantee of a successful implantation, pregnancy or other desired outcome. Rather, the intention of the tests is to increase the likelihood of a successful implantation.

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## INFORMATION

### Scientific evidence:

1. Evidence that the endometrial microbiota has an effect on implantation success or failure. Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almazán J, Alonso R, Alamá P, Remohí J, Pellicer A, Ramon D, Simon C. Am J Obstet Gynecol. 2016 Dec;215(6):684-703.
2. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology. Moreno I, Cicinelli E, Garcia-Grau I, Gonzalez-Monfort M, Bau D, Vilella F, De Ziegler D, Resta L, Valbuena D, Simon C. Am J Obstet Gynecol. 2018; 218(6): 602.e1-602.e16.
3. Endometrial microbiota composition is associated with reproductive outcome in infertile patients. Moreno I, Garcia-Grau I, Perez-Villaroya D, Gonzalez-Monfort M, Bahçeci M, Barrionuevo MJ, Taguchi S, Puente E, Dimattina M, Lim MW, Meneghini G, Aubuchon M, Leondires M, Izquierdo A, Perez-Olgati M, Chavez A, Seethram K, Bau D, Gomez C, Valbuena D, Vilella F, Simon C. Microbiome. 2022 Jan 4;10(1):1. doi: 10.1186/s40168-021-01184-w.

### Bibliography:

4. Guía Mensa: "Guía de Terapéutica Antimicrobiana 2022"; Autores: Mensa, J., Soriano, A., López-Suñé, E., Llinares, P., Barberán, J. y Zboromyrska, Y.
5. Sanford guide: "The Sanford Guide Antimicrobial Therapy 2022"; Autores: Gilbert, D., Chambers, H., Eliopoulos, G., Saag, M. y Pavia, A.

### SIGNED & COUNTERSIGNED



**Maria Ruiz,**  
02715-CV

General Supervisor  
Endometrial Receptivity Department

*This test has been outsourced to a referral laboratory (IGENOMIX Group)*

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#### LIST OF RECOMMENDED PROBIOTICS:

Whenever possible, the use of vaginal probiotics is suggested. In case they are not available in pharmacies and/or online shops, it would be to ask a pharmacist to prepare vaginal ovules or capsules containing at least one of the following Lactobacillus species: L. crispatus, L. iners, L. gasseri and L. jensenii in a minimum quantity of  $10^9$  CFU of each species. In this case, probiotic suppositories or capsules should be inserted pushing it high into the vagina (one every day for 10-12 days at bedtime).

WARNING: some probiotics contain progesterone in their composition. These probiotics are not included on the list, since they are not recommended in case of having performed an ERA or EndometRIO test, because they could alter the reproducibility of the results obtained.

#### VAGINAL PROBIOTICS:

##### EUROPE

Acidif CV. Biohealth. Vaginal tablets.  
Bactigyn. CCD Laboratoire de la femme. Vaginal suppositories.  
Canesflor o GineCanesflor. Bayer. Vaginal capsules.  
Dicoflor Elle Med. AG Pharma. Vaginal capsules.  
Gynexelle ProGyn. Bionike. Vaginal capsules.  
Gynolact vaginal tablets N8 Vitabalans. Gynolact. Vaginal suppositories.  
GynOphilus. Biose. Vaginal capsules.  
Invag. Biomed. Vaginal capsules.  
Muvagyn. Casen Laboratories. Vaginal suppositories.  
Normogin 40mg. Baldacci Laboratory. Vaginal tablets.  
Physioflor or Physioflor LP. IPRAD Laboratories. Vaginal capsules.  
Probio3 AH Vaginal. Tegor. Vaginal capsules.  
Probio Fem. Bonusan. Vaginal suppositories.  
SAUGELLA Attiva. MYLAN PHARMACEUTICALS. Gel and Vaginal capsules.  
Softigyn. Solartium Group. Vaginal capsules.  
Vagi-flora. Assos Pharma. Vaginal capsules.  
Vivagyn. Densmore. Vaginal capsules.  
Woman ISDIN Isadin Plus. ISDIN. Vaginal suppositories.

##### ASIA

AB-intimus. AB-Biotics. Vaginal tablets.  
Canesten Canesflor. Bayer. Vaginal capsules.  
Ginestra.Pharmaline. Vaginal tablets.  
Gynolact vaginal tablets N8 Vitabalans. Gynolact. Vaginal suppositories.  
Invag. Biomed (imported from Europa). Vaginal capsules.  
Lactoflora Forte. Sante Laboratory Co., Ltd. Vaginal capsules.  
Lactosupple. Eight Oh Trading company. Vaginal capsules.  
Physioflor. IPRAD Laboratories. Vaginal capsules.  
VagiBiom. Biom. Vaginal suppositories.

##### OCEANIA

Canesflor. Bayer. Vaginal capsules.

##### MIDDLE EAST

Dicoflor Elle Med. AG Pharma. Vaginal capsules.  
Purfem. Aralez Pharmaceuticals. Vaginal suppositories with applicator.

##### INDIA

Evanew vaginal tablets. Zuventus Healthcare Ltd. Vaginal tablets.

Another option could be to take oral probiotics, bearing in mind that treatments tend to be longer, as recolonisation of the reproductive tract occurs indirectly. Our recommendation is to use probiotics composed solely of Lactobacillus. Below is a list of some probiotics composed solely of species of the genus Lactobacillus.

#### ORAL PROBIOTICS:

Donnaplus Flora íntima. Ordesa. Oral capsules.  
Physioflor. IPRAD. Gastroresistant oral capsules.  
Seidivid Ferty4. Seid Lab. Oral capsules.  
Seidibiotics Plus. Seid Lab. Gastroresistant oral capsules.  
FertyBiotic Balance. Fertypharm. Oral capsules.  
Gynofit. Tentan AG. Oral capsules.  
Femelle. Bromatech. Oral capsules.  
Thera íntima. Theramex. Oral capsules.  
Ns Gineprotect Femibiotic. Cinfa. Oral capsules.  
GenMont. Biotech corporation. Oral capsules.  
Lactoflora intimate protection. STADA. Oral capsules.  
Vagimed. Lactopia. Oral capsules.

##### USA

VagiBiom. Biom. Vaginal suppositories.  
Vaginal probiotics composed of Lactobacillus only acquired at  
[www.rosemontpharmacy.com](http://www.rosemontpharmacy.com)

##### CANADA

Flora SAP. NFH. Vaginal suppositories.  
HMF Vaginal Health Probiotic Suppositories. Genestra/Seroyal. Vaginal suppositories.  
Provacare vaginal probiotic. Provacare. Vaginal suppositories.  
VagiBiom. Biom. Vaginal suppositories.

##### SOUTH AMERICA

Canesflor. Bayer. Vaginal capsules.  
Gynophilus. Synthon Chile. Vaginal capsules.  
Isadin a barcilus or Isadin Plus. ISDIN Laboratories. Vaginal capsules.  
Lactinex. Omega Laboratory. Vaginal suppositories.  
Purfem. Aralez Pharmaceuticals. Vaginal suppositories with applicator.  
Tropivag. Lukoll. Vaginal capsules.

##### MEXICO

Gynophilus. ELEA. Vaginal capsules.

